MEDITSINSKAYA RADIOLOGIYA
[Medical Radiology]

No 8

1959

19981203 098

MEDITSINSKAYA RADIOLOGIYA

TABLE OF CONTENTS

REVIEW

BRIEF COMMUNICATIONS

CRITIQUE

ANNALS

The Use of High Energy Radiation for Therapeutic Purposes

(Presented at the Eighth All-Union Congress of
Roentgenologists and Radiologists. Saratov, 1958)

M. P. Domshlak

Along with the γ-radiation of radioactive cobalt
(Co^{60}) extensively utilized at the present time in therapy
very hard braking (X-ray) and electron radiation generated
by means of betatrons of various designs and linear accel-
erators are becoming widespread. Experimentally and under
clinical conditions a study is being made of the efficacy of
the local effect of narrow beams of protons with energies
in the hundreds of millions of electron volts and α-parti-
cles with energies close to a billion electron volts (To-
bias and others). During recent years the possibility of
neutron therapy, which shows promise of exerting a really
selective effect of radiation (Farr and others: Brownell
and Sweet; Frigerio), has been persistently investigated
by several creative groups of physicians, physicists and
constructors.
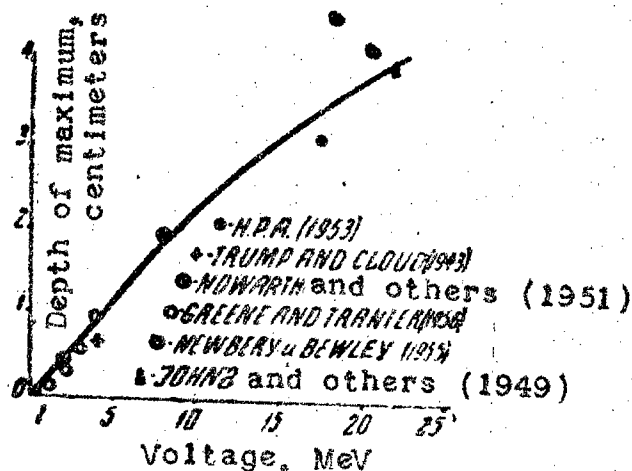
Fig. 1.   Shift of the Dose Maximum to the Depth of an Ir-
radiated Tumor Mass with Increase of Energy over
Four-Five MeV.

First of all, let us analyze the advantages and de-
fects of the use of braking (X-ray) radiation of megavolt
energies. The application of X-rays of megavolt energies
has the following advantages.

1. The maximum dose shifts notably into the depth
of the tissue mass and in the direction of the tumor being

irradiated (Fig. 1) with increase of energies above four to five MeV.

2. The dose falls off gradually from the maximum level. This makes it possible to affect the entire tumor mass uniformly.

3. The surface dose decreases and can be considerably less than the subsurface dose; this tends to remove the parasitic effect of the tissue lying in front of the tumor, primarily skin.

4. The lateral (and also back) scartering is very small. This in itself protects the healthy tissue surrounding the tumor. In addition, the possibility arises of keeping the beam within the tissue volume by giving the section of the beam the desired shape. The possibility of maintaining the shape of the beam of rays with high energy levels has opened up the way to the effective use of radiation through a grid, irradiation by the wedge method (Paterson; Becker). It is well known that scattering of ordinary X- and γ-radiation reduces the useful effect of the grid in the practice of deep radiation therapy.

5. With a low degree of back and side scattering the total-body dose received by the body is reduced, which decreases the total harmful effect of radiation.

6. Screening of the rays by bone is minimal. This increases the effect on the tumor located behind bony masses (skull, pelvis).

7. Injury of the bone and cartilages, the danger of which is well known in ordinary practice, is reduced to a minimum under conditions of hard radiation to a certain limit. This is connected with a lesser absorption of X-rays of megavolt energies by the calcium and sulfur atoms than in the case of ordinary X-rays with a smaller degree of difference of effect on the soft tissues, on the one hand, from that on bone and cartilage, on the other. Naturally, the organic bone substance is less injured under these conditions. Meredith points out the optimum level of radiation energy -- up to 20 MeV. The formation of ion pairs, which occurs after effects from energies of five to 20 MeV, does not have much influence, but at higher energy levels the fractions of energy absorbed by the bone increases again, the course of the secondary electrons increases, which creates the conditions again for the unfavorable effect on the soft tissues near the bone, the Haversian canals, and the organic bone matter.

In Fig. 2 the degree of uptake of X-radiation of different degrees of energy is shown in fat, muscle, cartilage and bone tissues. The uptake of ordinary X-radiation in bone tissue, as is seen in the left half of the graph, is considerable. Further on (middle portion of the graph) it may be seen

2

that radiation with energies of the order of several millions of electron volts is not much different with respect to absorption in various tissues.
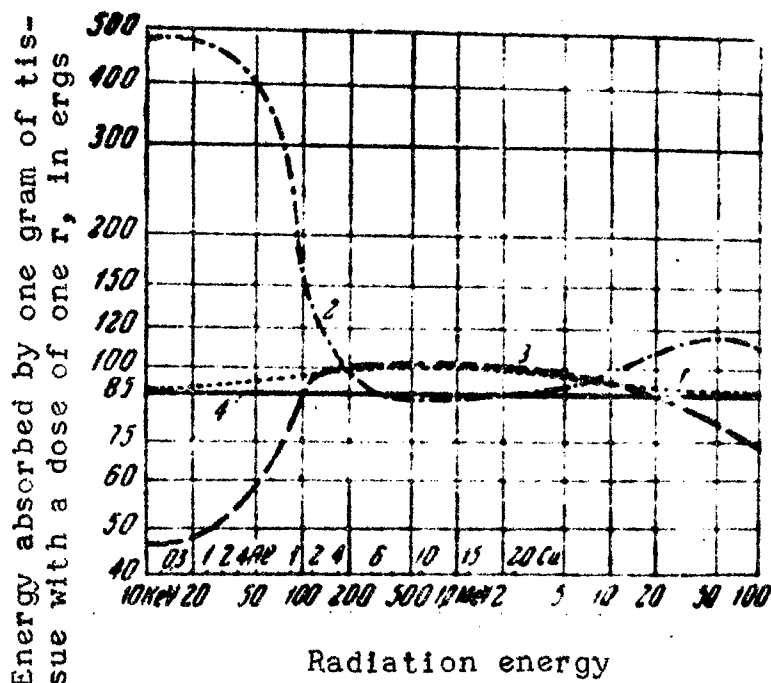
Fig. 2. Mass Absorption Coefficients of X-radiation with Energies up to 100 MeV.

1 -- muscle tissue; 2 -- bone tissue; 3 -- fat tissue; 4 -- air.

With a further increase in the energy the absorption in bone again increases because of the effect of the formation of ion pairs, which is reflected in the right-hand portion of the graph. A level of 5 to 20 MeV is considered optimum. At the same time, very hard radiation is not free of defects in a therapeutic sense.

1. Along with the increase in energy the exit dose increases. This increase cannot always be neglected. The exit dose notably exceeds the entrance dose with energies of braking radiation over 25 MeV. Within limits of three to 10 MeV apparently the best conditions are created with respect to the skin dose (Fig. 3).

2. With the use of very hard radiation the usual index of the reaction to irradiation of the skin is lost, and the danger arises of underestimating the reaction of deep tissues. This is not of importance in principle; however, particularly at the beginning of familiarization with

3

the method it may be of great practical importance.
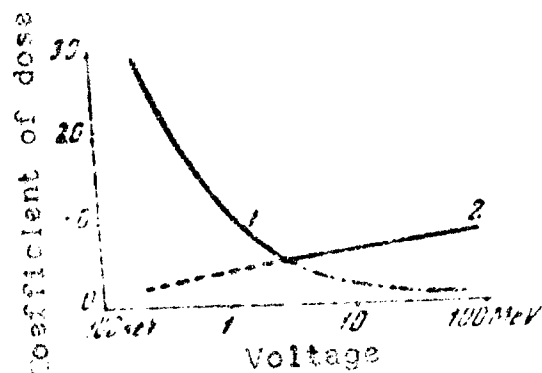
Fig. 3. Relationship of Entrance and Exit and Doses with Different Energies (Under 100 MeV) of X-radiation.

1 -- entrance dose; 2 -- exit dose. Coefficient of en-

$$\text{trance dose} = \frac{\text{surface dose}}{\text{dose at a depth of 10 centimeters}}.$$ Coeffi-

$$\text{cient of exit dose} = \frac{\text{dose at depth of 20 centimeters}}{\text{dose at depth of 10 centimeters}}.$$

3. Because the dose decreases slowly in the depth o the body from the area of the maximum overirradiation of th tissues around the tumor is possible. This may be avoided by using several fields (Paterson; Tubiana and others). Paterson in his practice of irradiation with energies of fc to five MeV found a solution in theuse of no less than thre fields with a special technique of irradiation.

Betatrons and linear accelerators, including the con paratively compact and movable betatron for 15 MeV and the variant of the betatron for 32 MeV with a traveling beam are used as sources of the rays of megavolt energies. Pro- gress made recently in the manufacture of linear accelera- tors makes them, certain authors believe, the most conven- ient sources of retarding the simultaneous electron radi- ation at 3 to 10 MeV.

From the data presented above the conclusion may be drawn that generators of hard braking (X-ray) radiation of three-eight MeV are desirable for therapeutic purposes. Only with the accomplishment of further comparative experi- mental and clinical investigations will it be possible to say for sure that the use of high energies is expedient. The original data gave rise to an overestimation of the po: sibilities of irradiation with very high energies. This stimulated the creation of 24 MeV betatrons (Allis-Chalmer:

4

and 31 MeV betatrons (Brown-Boveri). At the present time, this view is being revised in the light of the data obtained.

γ-apparatuses with radioactive cobalt are also soures of megavolt radiation, because the Co^{60} radiation corresponds to the continuous spectrum of a three-megavolt X-ray apparatus. The problem of comparative values of the various sources of radiation for purposes of therapy is debatable. Based chiefly on the data of the Manchester School a comparison of their merits and deficiencies may be made in the following way.

Gamma Apparatuses with Co^{60} Source	Linear Accelerators for Four MeV and More
Merits	
Does not require any high voltage apparatus -- radiation generators. Constant character of radiation. Simplicity of control.	Larger subsurface dose (telegramma-apparatus with Co^{60} creates a maximum dose at a depth of about six millimeters; linear accelerators -- at a depth of several centimeters). Small skin dose. Little effect on the bone. Small half-shadow (five millimeter focal spot). High dose rate (120 4/min) at a distance of one meter with four MeV. Great output (12 fields in an hour. Experience in its use has shown a very constant output of retarding radiation and simplicity of operation.
Defects	
Necessity of replacing the Co^{60} preparations in the apparatus. Large dimensions of the source, large half-shadow. Comparatively small dose rate (40 r/min) from 2000 curies of Co^{60} with a skin-to-tube distance of one meter.	Rigid housing requirements. High cost.

It seems unjustified, in any case at present, to draw mutually exclusive conclusions in the comparison. A striving

toward universalism in the technique of irradiation has no
sound basis. Our own experience and the recent discussion
at the Second International Conference on the Peaceful Uses
of Atomic Energy (Paterson; Brucer and Simon; Baarli) con-
vince us of the expediency of further use and further develop-
ment of new designs of gamma-apparatuses of the short-focus
(plesiotherapeutic) and long-focal distance (teletherapeutic)
types. At the same time, possibilities of utilizing rays,
new in principle, with energies greater than in Co^{60} stimu-
late us to carry out works on the application of linear ac-
celerators and betatrons.

Within the framework of the present report it is im-
possible to dwell in detail on the problems of the indica-
tions for the use of high-energy radiation and the results
obtained in the preliminary investigations (Becker, Tubiana
and others; Schinz, Zuppinger; Oberheuser and Schmermund).
Nevertheless, it should be pointed out that the indications
for the use of the new technique are great. Advantages of
irradiation of tumor recurrences which have occurred in sur-
face tissue layers previously irradiated and already injured
may be noted (Zuppinger). It should be taken into consider-
ation that initially the indications were limited to the area
of palliative radiation therapy; then, the dosage was refined,
the method perfected, and experience accumulated. To date
the optimum doses and methods of irradiation are still being
worked out. Therefore, the results are contradictory and un-
convincing. A review given in 1958 (Becker) shows progress
made in treating brain tumors, tumors of the esophagus,
stomach, bladder, female sex organs and others along with
contradictory data. Reports concerning associated changes
in the tissues deserve attention. At the same time, in the
Heidelberg Radiological Hospital (Becker) no complications
were observed. Here, use was made of a 15-megavolt betatron
with a grid and a movable ("pendulum") irradiation. The
author believes that injuries did not occur because the
total dose did not exceed 6000 r with daily irradiations of
200 r.

Oberheuser and Schmermund indicate the advantages of
very hard braking radiation with respect to cancer of the
genitalia. Whereas by means of ordinary apparatuses and the
use of movable irradiation large depth doses may be created
only within a limited dosage maximum, theuse of a 16-mega-
volt radiation made it possible for the authors to achieve
the same effect with respect to a large tissue volume with
an effect on the lymph outflow tract.

Electron irradiation is theoretically and practically
very similar to the method of very hard braking (X-ray) ir-
radiation. High energy electrons create a maximum dose in

tissues at a depth of from several millimeters to one to two centimeters. By changing the electron energy this depth maximum may be shifted within certain limits (practically up to 10 MeV). In Fig. 4 the distribution of energy of a 22-megavolt electron beam in irradiated tissue is shown by comparison with a distribution of a 400-kilovolt X-radiation (Δ = 2.75 millimeters of Cu, with a field of 100 square centimeters, a skin-to-tube distance of 50 centimeters) and a 22.5-megavolt braking (X-ray) betatron (field of five centimeters in diameter and a skin-to-tube distance of eight centimeters). The markedly limited depth distribution of the beam may be seen.

Fig. 4. Comparative Distribution of Electron and X-radiation Energy.

1 -- electrons (22 MeV);
2 -- X-rays (400 keV);
3 -- X-rays (22.5 MeV).

For certain indications electron beam therapy can apparently be preferable. Existing data (Oberheuser and Schmermund; Haas and others; Skaggs and others) make it possible to evaluate certain possibilities and results of treatment. Apparently, electron therapy is most indicated when tumors are localized in the nasal sinuses, the oral cavity, the pharynx, larynx, with cancer metastases to lymph nodes, with recurrences of breast cancer in the postoperative scar, in primary skin cancers and malignant melanomas, in tumors of the thyroid gland and tumors of the external sex organs. In electron therapy use is made of irradiation through a grid and intracavitary irradiation through tubes.

Skaggs and coauthors have recently reported a further improvement in the technique of electron therapy. The apparatus which they developed makes it possible to regulate the energy of the electrons from 5 to 50 MeV, which makes it possible to irradiate with a greater or lesser maximum depth dose. It makes it possible also to give any shape to the transverse section of the beam, to shift the beam in various directions, including rotation up to 360°. This apparatus may be utilized in working with various electron accelerators.

We believe that the possibility of creating a plateau of the dose maximum from the surface being irradiated to a certain depth (up to two centimeters) constitutes an advantage of electron therapy of tumors which are directly accessible to this type of radiation. In the laboratory

at Berkeley 900-megavolt α-particles are used by means of a 184-inch cyclotron. A considerably collimated, narrow beam of α-particles which scatter very little and are of high energy levels are sent into a very small tissue volume of a size appropriate to the irradiation, for example, one cubic centimeter. Because the α-particles possess a definite path length their effect may be concentrated within limits of the volume being irradiated at the end of the path of the particles, which tissues lying on the route of the beam remain practically uninjured. The object of such a strictly directed effect in the Berkeley investigations was mainly the hypophysis. At the present time, valuable data have been accumulated pertaining to radiation hypophysectomy for disseminated breast cancer (Tobias and others). In this case the possibility of a strictly directed and limited effect is utilized clinically (and experimentally) for elucidating the role of the hormonal factor in the pathology of breast cancer. Undoubtedly both for clinical radiology and for radiobiological investigations the method which is being developed for using high energy corpuscular radiation is of great importance, transcending the boundaries of the problem being studied at Berkeley.

The striving for the most advantageous distribution of energies, the maximum effect on the tumor with the minimum irradiation of surrounding tissues and of the body as a whole remains a goal which is far from being reached. One of the most fortunate approximations to the solution of the problem of the selective irradiation of tumors is the method of neutron-capturing therapy which is now being developed experimentally and clinically. The idea underlying the method arose immediately after the discovery of neutrons and the obtaining of sources of neutron radiation (Brownell and Sweet). It consisted of the creation of induced activity in a tumor saturated with some substance with an adequately large area of capture of thermal neutrons. There are great difficulties in the way of carrying out this idea. They apply chiefly to the creation of adaptations for an adequately intense irradiation with thermal neutrons, to finding substances which satisfy the requirement of maximum capture of neutrons and at the same time are without toxic properties, to the development of efficient methods of saturating the tumor with these substances.

Of the substances which may be used in this method, which has obtained the name of selective kinetics, there are boron (B^{10}), lithium (Li^6) uranium (U^{235}). The best developed method is that based on the use of B^{10}, a stable isotope present in natural boron to the extent of 18.8 percent. The greater rate of passage of boron from the blood into the tumor

tissue than into the surrounding tissue is utilized.

Clinical tests have been made with the irradiation of seriously ill patients with blioblastomas. With an infiltrative growth of these tumors the hemato-encephalic barrier in their area is disturbed, which also creates an accelerated passage of substances circulating in the blood into the tumor tissue, particularly of B^{10}.

First, borax was used -- the decahydro sodium salt of tetraborate solution was injected through the antecubital vein. The maximum difference of concentration of boron between the tumor and brain tissue is created 10 minutes after the injection. Afterwards, a decrease in the concentration occurs over the course of an hour. During the period of maximum saturation of the tumor with boron neutron irradiation was carried out by means of an adaptation for the reactor which was created. As a result of the interaction of neutrons with boron in the tumor cells α-radiation arises and acts on them:

$$B^{10}_5 + n \rightarrow Li^7_3 + \alpha$$
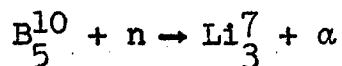
These α-particles are distributed within limits of 14μ, which corresponds to the cellular dimensions and limits their effect on the healthy tissues. The beam of $3 \cdot 10^8$ neutrons per square centimeter per second proved to be small and was reduced to 10^{10} n/square centimeter $\cdot$ sec. Encouraging palliative results were obtained. However, the efficacy of treatment remained inadequate as the result of the toxicity of boron in the large doses needed, which were required in accordance with the calculation made for creating a carcinolytic effect. In addition, complications were observed in the form of necrosis of the skin in areas of neutron irradiation.

After wards, it was possible to increase the quantity of boron injected and to increase the average number of hot neutrons which could be passed through the skin fields. The method itself was changed. Sodium tetraborate was replaced by pentaborate, $Na_2B^{10}O^{16}$, which, as was made clear, is less toxic. Now pentaborate perfusion began to be used with the latter being injected into the internal carotid artery on the side of the brain lesion, which provided a greater concentration of boron in the tumor. Here, irradiation was accomplished during almost the entire time of perfusion. This method of perfusion was accomplished by a specially created apparatus. In order to avoid skin injuries the time of action of the neutron beam had to be limited to 10-11 minutes after the beginning of the injection of the boron, because

later a side effect of the reaction of neutron capture could occur in the skin tissue. This poses the problem of utilizing sources of more powerful thermal neutron beams, of the order of 10^{12} n/cm^2 · sec. The method developed provides for the maintenance of a level of boron solution with perfusion under the control of the rate of the cerebral blood flow and with consideration of the perfusion time so that the dose of boron does not exceed the permissible level. The rate of injection of the boron is controlled by means of fluorescein in ultra-violet light. Clinical tests of the new modification of the method of neutron-capturing therapy have shown that the intracarotid injection is not accompanied by any complications, skin lesions do not occur if the boron is injected in the course of 10 minutes, and neutron irradiation does not exceed 12 minutes from the start of boron injection. The experimental data obtained in animals show that the increase in the dose rate of the neutron beam and in the quantity of boron injected may suppress the tumor growth to quite a high degree.

Recently, a study has been made of the possibility of utilizing various nuclides which are applicable as substances with epithermal capture peaks (Brownell and Sweet). Apparently, this possibility is very much limited by the need for injecting large quantities of similar substances into the body and the condition of the high epithermal flux. Brownell and Sweet see a further means of overcoming the difficulties in the direct irradiation of tumor tissue or in improving the distribution of the thermal neutrons by means of introducing epithermal neutrons into the beam. Therefore, the method of neutron-capture therapy is being studied in all of its aspects: physical, experimental-biological (toxicology of capturing substances, morphological study of tumors and normal tissues exposed to irradiation) and clinical.

The possibility of utilizing epithermal neutrons, which is well known according to the text of Frigero's report at the Second International Conference on the Peaceful Uses of Atomic Energy, is of particular interest. This possibility consists, for example, in an epithermal activation of iodine in tumors of the thyroid gland and its metastases resistant to other effects. Natural iodine (I^{127}) vigorously absorbs neutrons with energies of from 20 to 200 eV. As the result of activation I^{128} occurs -- the β-emitting isotope with an energy of the β-particles of two MeV and a short half-life period (T = 25 minutes).

Many tumors of the thyroid gland do not possess the capacity for a high degree of I^{131} accumulation; at the same time, they contain adequately stable iodine. In similar cases the epithermal activation of iodine can prove to be a useful

method which is devoid of any radiation side-effects of the isotopes injected into the body in a state which is already radioactive. The advantage of this method by comparison with the use of thermal neutrons is the fact that side effects of radiation occurring from thermal activation in such widespread nuclides of the body tissues as hydrogen, nitrogen and chlorine are eliminated.

The development of new trends of radiation therapy is certainly associated with the elucidation of the relative biological efficacy of high energy radiation, which is of essential importance for clarifying the levels of the optimum doses. We will not concern outselves with this problem. Certain data pertaining to the relative biological efficacy of megavolt energy radiation have been presented in our recent publication (M. P. Domshlak, N. G. Darenskaya).

In evaluating the importance of the new possibilities of radiation therapy being analyzed we should not overlook the achievements of surgery and chemotherapy or, incidentally, of the other aspects of radiotherapy (the use of radioactive isotopes of gold, iodine, phosphorus and others). The arsenal of substances used in modern associated and combined therapy of malignant tumors has become powerful, and the agents which have now become familiar have created a strong basis for the further even more successful development of means of overcoming the malignant growth.

When, almost 50 years ago, the high-voltage transformer replaced the magneto it was a very great event, which laid the basis for the rapid development of roentgenology. A quarter of a century ago progress in radiobiology created a rational basis for radiation therapy. Now, new possibilities of radiation therapy are being realized. However, this realization requires great measures. First of all, it is necessary to include physicists and constructors in research and practical work (in the radiological clinic). Another condition should be the developing of experimental and clinical radiology at large combined hospitals (clinical towns). The third condition, in our opinion, consists of a revision of the training of radiological specialists. These and other conditions needed for the successful utilization and development of medical radiology are being provided in part. The problem reduces itself to the rapid and effective accomplishment of scientific-organizational solutions.

Bibliography

Volkova, M. A.; Domshlak, M. P.; Shirokov, S. I. "Vrach. delo" [Physician's Affairs], 1945, Nos 9-10, page 458.

Domshlak, M. P. "Med. radiol." [Medical Radiology], 1956, Vol 1, No 2, page 10.

Domshlak, M. P.; Darenskaya, N. G. In the book: "Radiobiology," Moscow, 1957, page 149.

Nevskaya, G. F. "Vestn. rentgenol. i radiol." [Herald of Roentgenology and Radiology], 1959, No 2, page 63.

Baarli, J. Second United Nations International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958. Paper 579.

Becker, I. "Strahlentherapie," 1958, Bd. 106, S. 85.

Brownell, G. I. Second United Nations International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958. Paper 881.

Brucer, M.; Simon, N. Second United Nations International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958. Paper 2403.

Farr, L. E.; Robertson, J. S.; Stickley, E. E. and others. Ibid. Paper 883.

Haas, L. L. and others. "Am. J. Roentgenol.," 1954, Vol 79, page 250.

Meredith, W. J. "Am. J. Roentgenol.," 1958, Vol 79, page 57.

Oberheuser, F.; Schmermund, H. I. "Strahlentherapie," 1958, Bd 106, S 127.

Paterson, R. Second United Nations International Conference on the Peaceful Uses of Atomic Energy. Geneva, 1958, Paper 67.

Skaggs, L. S.; Lanze, L. H.; Avery, R. T. Ibid. Paper 904.

Schinz, H. R. "Fortsch. Geb. Rontgenstrahlen," 1957, Bd 86, S 104.

Tubiana, M.; Dutreix, J.; Pierquin, B. and others. "J. radiol. et electrol.," 1955, Vol 36, page 507.

Zuppinger, A. "Radiol. Clin.," 1955, Vol 24, page 65.

Idem. "Med. et hyg.," 1955, Vol 229, page 1.

The Investigation of Reception of Irradiated Areas of the Body in Experiments on Animals

N. S. Delitsyna

Our previous report was devoted to a study of the re-
action of the cerebral cortex in response to touch stimula-
tion of the irradiated area in people exposed to the effect
of X-rays for therapeutic purposes. In attempting to come
nearer to a discovery of the mechanisms of the phenomena
shown we continued these investigations under experimental
conditions.

The experiments were performed on 30 males rabbits
weighing 2300-3000 grams.

The animals were exposed to a single local effect of
filtered (0.5 mm Cu and one mm Al) of X-rays either on the
area of the foot (20 rabbits) in a dose of from 500 to 5000
r (tube 4 x 4 centimeters).

Investigation of the touch stimulation was carried
out by means of stroking the skin of the leg with a brush
during the recording of the action currents of the parietal
area of the cerebral cortex. The action currents were fed
into an amplifier assembled according to a push-pull system
with a symmetrical input and feed from an alternating cur-
rent system. The recording of the action currents was ac-
complished by means of a loop oscillograph on movie film.

In animals exposed to a local irradiation in a dose
of 500 r the reaction of the action currents of the cortex
to touch stimulation of the irradiated area was very simi-
lar to what we observed after local irradiation of people.
While the changes in electrical activity of the cortex in
response to touch stimulation were weak in these animals
before irradiation, immediately after the irradiation an ex-
acerbated reaction occurred which began to weaken only af-
ter one to three days. However, even seven days after the
effect the reaction of the action currents remained intensi-
fied with a reproduction of the rhythm of the touch stimu-
lation.

A marked change in the cerebral cortical reactivity
in response to touch stimulation can be seen from stimula-
tion not only of the irradiated areas but also of areas at
a considerable distance from the site of the direct effect
of radiation energy. The reaction produced by stimulation
of these areas is always less distinct than from the area
of irradiation.

No signs of radiation sickness were seen in the exper-
imental animals; their behavior remained as usual, their
appetite was good; there were no temperature or weight vari-

ations.
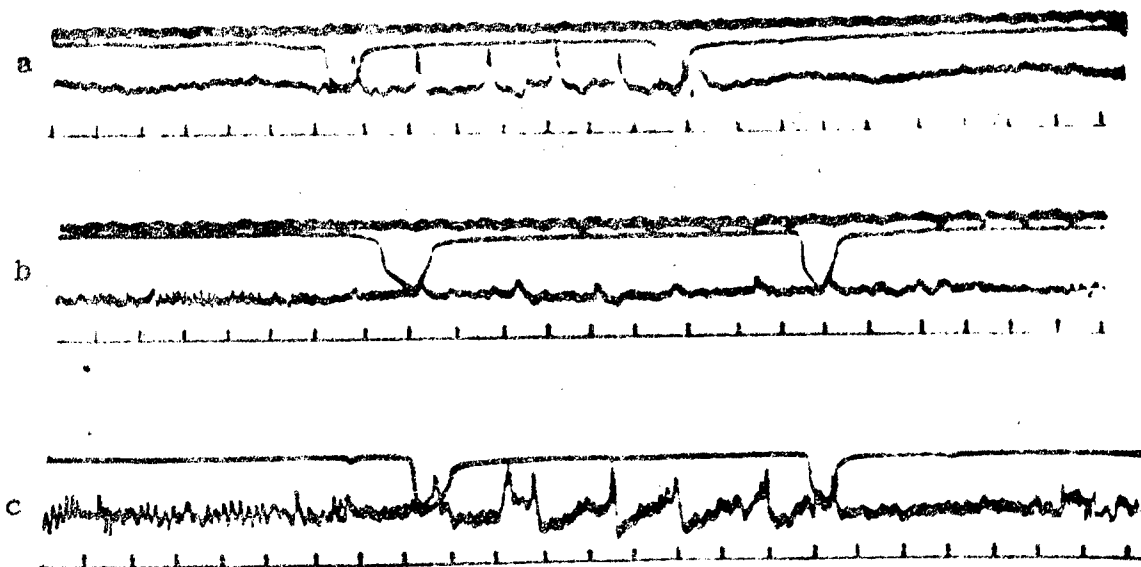
Electroencephalogram of the Parietal Region of the Cortex
of a Rabbit Upon Tactile Stimulation of the Shin.

a -- before irradiation; b) 30 minutes after irradiation;
c -- two days after irradiation. From top to bottom: pneu-
mogram; tracing of tactile stimulation; electroencephalo-
gram; time in seconds.

The cerebral cortical reaction was of a different
nature in rabbits in response to touch stimulation after
a local irradiation of the leg or foot with filtered X-
rays in a dose of 5000 r (see Figure). Ten to 15 minutes
after irradiation the changes in the cortical action cur-
rents produced by stimulation became weaker, although they
maintained their previous character, with a smaller ampli-
tude (b). Two days after the irradiation the reaction in-
creased markedly (c). Here, not only the degree of the
changes produced by stimulation increased but the nature
of these changes was also altered. Individual touch stim-
ulation was now associated with a discharge of fast waves
of cortical action currents which subsided quickly after
stopping the stimulation.

Afterwards, one to four weeks after the irradiation,
an instability of the cortical reaction to touch stimula-
tion was observed: at times, there was a weakening of it
up to complete extinction and at other times a marked
exacerbation. In two rabbits with a predominance of a
stable rhythm in the action currents, which reflected the

14

frequency of respiration, the reaction of the action currents to touch stimulation was expressed through the medium of these changes in cortical rhythm.

In experiments on animals, just as in observations made on people, changes could be observed in the electrical activity of the cortex in response to touch stimulation not only of the irradiated area but also of parts of the body at some distance from the site of the direct radiation factor effect.

In animals of this group both before and after irradiation a measurement was made of the motor chronaxie of the flexor and extensor muscles of the appropriate extremity. The investigation of the motor chronaxie of the muscles of the irradiated extremity showed a tendency toward equalization of the chronaxie of the antagonistic muscle groups through an increase in the chronaxie of the flexors and a reduction in the chronaxie of the extensors during the first one or two days after the radiation effect. Then, these changes acquired an irregular, abrupt nature and disappeared only one to one and a half months after irradiation. Because similar changes were noted also in the contralateral extremity which had not been exposed to a direct effect of X-rays apparently the main cause of these changes is the presence of subordination influences from the central nervous system.

After irradiation the rheobase changed in all of the cases which we observed with the exception of one. These changes were sometimes marked but it was impossible to find any definite rules and regulations in them.

In animals exposed to local irradiation of the foot with X-rays in a dose of 3000-5000 r an extensive lesion of the skin developed in time, first on the irradiated extremity and then also on the opposite side of the body in the non-irradiated, contralateral extremity.

Three to five days after the irradiation and while the animal was in good condition an area of epilation appeared at the site of irradiation. At this site the skin was first dry, thinned out and desquamating; then, erythema appeared around which a dense infiltrate occurred. In the center of the focus a small crust appeared, and afterwards an extensive sloughing of the epidermis occurred. The necrotic tissue peeled off in whole layers, exposing a delicate pink, poorly granulating ulcerated surface with a small quantity of serous exudate. Despite attentive care the ulcer rapidly became infected, and the discharge acquired a purulent nature.

Four to six days after the appearance of epilation on the irradiation side exactly the same kind of area ap-

peared in the opposite side of the body in the contralateral extremity. It underwent the same changes as on the side exposed to the direct effect of radiatn energy but with a smaller area of the lesion.

The general condition of the animal at this time deteriorated markedly; sluggishness, adynamia appeared, and the temperature rose, the appetite decreased, the body weight dropped, and a liquid stool was sometimes noted. These signs were observed for 7 to 10 days.

Irradiation of the leg produced a smaller degree of involvement. Healing of the ulcers on the irradiated extremity occurred after two and a half to three and a half months in the majority of cases. In the opposite side of the body in the contralateral extremity an epilation occurred with subsequent maceration of the epidermis and the appearance of a weeping surface.

Healing began earlier and progressed more rapidly in extremities which had not been exposed to irradiation. At thesite of the ulcers there remained a smooth shiny white scar without the recovery of the hairy integument. In two rabbits after irradiation of the left foot and ulcerative lesion of all four extremities developed in sequence. After irradiation of the foot (500 r) the process terminated in a complete sloughing off of the latter in the majority of cases. The duration of the observation of the animals was four to five months.

At autopsy of the animals which died extensive hemorrhages in the internal organs were found in two rabbits with local irradiation of the foot (5000 r).

In the light of the data obtained it is interesting to mention the well known case of Becquerel. During his visit to England Becquerel kept an ampule containing radium in the pocket of his vest. After a certain time he accidentally found a burn on his chest (at a level corresponding to the location of his pocket). After several days, the spot occurred also on the opposite side of the chest, although there had been no radium ampules there.

S. V. Gol'dberg noted a similarity between the effect of radium preparations on the skin and X-rays. The development of the process proceeded differently in the case of S. V. Gol'dberg who carried out radium experiments on himself. The foci of the primary lesion occurred at the site of application of the radium preparation to the forearm, and then the same focus appeared in the lower portion of the arm (near the elbow flexure); later, in the area of the left inguinal fold and on the left side of the chin. All the foci of involvement, with the exception of the last one, acquired the nature of ulcers in time. The healing

proceeded very slowly and in an order opposite to the order of occurrence of the foci. The ulcer which arose at the site of application of the radium preparation, on the other hand, continued to increase in size, and this self-sacrificing investigator died as a result of a carcinomatous degeneration of the indolent ulcer at the site of the burn.

The data which we obtained on animals coincide with the observations on people and attest to the presence of changes in the peripheral nerve-receptor apparatuses of the parts of the body which were subjected to the direct effect of the radiation factor.

Immediately after irradiation it was possible to record changes in the reaction of the highest central nervous system center -- the cerebral cortex.

A change in the functional condition of the central nervous system leads to a completely different reaction of the cerebral cortex to touch stimulation of the area of the body at some distance from the site of irradiation and particularly symmetrical with it from that which occurs before irradiation. A change in motor chronaxie not only of the irradiated but also of the contralateral non-irradiated extremity also attests to a change in the subordination (central) effects at the periphery.

With an adequately large irradiation effect (dose of 5000 r) the further development of a pathological process is manifested by an involvement of the extremities which were not exposed to irradiation as well as by hemorrhage in the internal organs. The fact that the involvement of the skin both on the side of the direct effect of the radiation factor and in areas of the body not subjected to the effect passed through the same stages of development and had exactly the same clinical form attests to the similarity of the mechanisms determining the development of the process in both cases.

The development of pathology of a segmental type convincingly confirms the fact that a neural mechanism underlies the process.

Venous Pressure Changes in Acute Radiation Sickness in Rabbits

A. S. Mozzhukhin

From the Military Medical Order of Lenin Academy
imeni S. M. Kirov

In acute radiation sickness a number of hemodynamic
changes are observed which consist of a decrease in arterial
pressure (Prosser, P. D. Gorizontov, A. S. Mozzhukhin and I.
A. Peymer and others), a reduction in the quantity of circu-
lating blood (Storey, G. M. Frank and others), a reduction
in the minute and systolic volumes of the blood (N. V. Bu-
tomo and others) and a change in the tone of the arterial
blood vessel walls (P. D. Gorizontov, G. M. Frank, N. V.
Butomo and others). There are grounds for the belief that
these changes in the hemodynamics may play a definite part
in the development of the hemorrhagic tendency in acute ra-
diation sickness (N. V. Butomo and others, T. K. Dzharak'yan
and A. S. Mozzhukhin).

Since it is well known that bleeding in acute radiation
sickness is observed chiefly from small veins (N. A. Krayev-
skiy, A. D. Smirnov and Z. P. Kovtun and others), the hemo-
dynamic changes and primarily the blood pressure in these
blood vessels are of particular interest. At the same time,
there is practically no information in the literature con-
cerning the nature of changes in venous pressure at different
periods of acute radiation sickness in different parts of
the venous system. P. D. Gorizontov mentions the irregular
variations in pressure in the superficial veins of the ex-
tremities of irradiated dogs. Armstrong and coauthors report
an increase in the venous pressure in the veins of the mesen-
tery in rats during the period of the primary reaction and
during the climax of acute radiation sickness.

Data in the literature concerning the nature of the
blood pressure changes in acute radiation sickness are essen-
tially limited to this information.

We undertook an investigation of the changes in ven-
ous pressure in different parts of the venous system of ir-
radiated animals.

The experiments were performed on male rabbits weigh-
ing two to 3 1/2 kilograms. The irradiation was accomplished
on an RUM-3 apparatus from the back in wooden boxes which
limited the movements of the animals. The following were
the irradiation conditions: voltage 180 kv, current 15 ma,
filter 0.5 mm Cu and 1 mm Al, half-value layer equal to 1 mm
Cu, distance from anode to center of the body 70 centimeters;

dose rate 10 r/min, total dose 800 r.

Over the course of two weeks before the irradiation and one month after the irradiation observations were made of the clinical condition of the animals, the white blood count was taken once every five days and the temperature and body weight were determined daily. The degree of severity of radiation sickness and its periods were determined by the change in these indices as well as by the data of autopsies. Under these experimental conditions the death of 50 percent of the animals was observed during the climax of the disease, leukopenia reaching 500 cells per cubic millimeter in the majority of animals, adynamia, refusal of food, disorder of gastrointestinal activity and other signs of acute radiation sickness.

The venous pressure was determined in acute experiments without anesthesia in veins which belong to three venous systems: 1) the superior vena cava system (marginal vein of the ear and external jugular vein); 2) the inferior vena cava system (inferior vena cava and femoral vein); 3) the portal vein system (portal and superior mesenteric veins). The pressure was determined by means of inserting needles into the vein connected with a mercurial manometer. This method is less accurate than the determination of venous pressure by means of a water manometer according to the Val'-dman method (1928, 1947), since it gives rise to an error of one to two millimeters in the mercury column. However, in our experiments it was more advantageous, because under these conditions less blood enters the connecting system. Therefore, blood coagulation occurs rarely in the system, which makes it possible to carry out all the measurements for 5 to 10 minutes. The short duration of the experiment reduces pressure changes to a minimum during the course of the experiment, which is of importance in determining the pressure in the veins of internal organs. On the other hand, the shortest duration possible is exceptionally important in performing experiments during the climax of acute radiation sickness, because the animals not uncommonly die during the courseof the experiment.

The experiments were performed on 30 rabbits (three groups of 10 animals each). The rabbits of the first group were not exposed to irradiation and served as controls. In the second group the venous pressure was determined during the period of the primary reaction (first day after irradiation); in the third group, at the climax of acute radiation sickness.

The results of the experiments are presented in the Table.

Venous Pressure Change Following Total-Body Irradiation of Rabbits with X-rays in a Dose of 800 r (Average Figures from 10 Experiments)

Vein	Control				Period of Primary Reaction (1st-2nd Day after Irradiation)				Climax of Sickness (7th-14th Day after Irradiation)			
	Pressure mm of Hg Systolic	Diastolic	Average	Mean Error	Pressure mm of Hg Systolic	Diastolic	Average	Mean Error	Pressure mm of Hg Systolic	Diastolic	Average	Mean Error
External Jugular.....	8	2	4	0.6	8	1	2	0.09	8	1	3	0.6
Marginal vein of the ear.......	50	14	27	3.6	30	1	9	2.5	40	4	16	3.6
Femoral.......	20	6	10	1.5	10	1	4	0.8	8	1	4	0.6
Inferior vena cava...	8	4	5	0.6	8	1	3	0.7	4	1	2	0.3
Superior mesenteric.	30	12	19	1.5	12	6	10	0.8	14	4	9	0.8
Portal.......	24	6	13	1.4	12	2	8	1.1	10	2	6	0.9

From the material presented in the Table it follows that during the period of the primary reaction and during the climax of the sickness a reduction in venous pressure is observed in all the venous systems. A statistical treatment shows that the decrease observed may be considered reliable (probability from 0.960 to one), that is, the difference between the mean pressure in the corresponding veins in the control and irradiated animals in all cases exceeds twice the average error, and in the great majority of cases even three times the error. During the period of the primary reaction the principal decrease in pressure is observed in the portal and superior vena cava systems, while during the period of the climax of the disease, in the portal and inferior vena cava systems. The data obtained attest to a marked reduction in the venous tone (particularly of the internal organs) in acute radiation sickness, because the decrease in venous pressure is combined with overfilling of the veins of internal organs, which is distinctly seen from autopsy (N. A. Krayevskiy, A. D. Smirnov and Z. P. Kovtun).

The reduction in the venous tone may be associated both with the morphological changes in the veins and with the neuro-humoral influences on the venous system. In conjunction with I. A. Peymer we showed a disappearance and inversion of the pressor carotid sinus reflexes during the period of the primary reaction and during the climax of radiation sickness; according to the data of Donegan and Fleisch, the venous tone in the abdominal cavity is maintained principally from these reflexes. The influence of active humoral substances described by P. D. Gorizontov and co-workers on the vein wall cannot be excluded. Whatever the mechanism of change in the tone of the vein wall and in the venous pressure these changes may play a definite part in the development of the hemorrhagic syndrome of acute radiation sickness along with other hemodynamic changes.

Conclusions

1. In experiments on rabbits irradiated with X-rays in a dose of 800 r a statistically reliable reduction in venous pressure was found in all irradiated parts of the venous system during the period of the primary reaction and during the climax of acute radiation sickness.

2. The marked distention of the vein walls in internal organs observed simultaneously with a reduction in venous pressure and attributable to overfilling of the veins with blood is evidence of a considerable reduction in the vein wall tone chiefly in internal organs during acute radiation sickness.

Bibliography

Butomo, N. V. and others. Proceedings of the All-Union
    Scientific Technical Conference on the Use of Radio-
    active Isotopes in Medicine. Moscow, 1957, page 92.
Val'dman, V. A. Blood Vessel Tone and the Peripheral Circu-
    lation. Leningrad, 1928.
Val'dman, V. A. Venous Pressure and Venous Tone. Lenin-
    grad, 1947.
Gorizontov, P. D. In the book: "The Biological Effect of Ra-
    diation and the Clinical Aspects of Radiation Sick-
    ness." Moscow, 1954, page 107.
Gorizontov, P. D. In the book: "Radiobiology." Moscow,
    1958, page 37.
Dzharak'yan, T. K., Mozzhukhin, A. S. Proceedings of the
    Conference on Medical Radiology Devoted to the For-
    tieth Anniversary of the October Socialist Revolution.
    Moscow, 1957, page 18.
Krayevskiy, N. A. Outlines of Pathology of Radiation Sick-
    ness. Moscow, 1957.
Smirnov, A. D.; Kovtun, Z. P. Proceedings of the Scien-
    tific Conference on Radiobiology Devoted to the 250th
    Anniversary of Leningrad. Leningrad, 1957, page 44.
Frank, G. M. In the book: "Effect of Radiation on the Body."
    Moscow, 1955, page 112.
Donegan, A. I. F. "J. Physiol.," 1921, Vol 55, page 226.
Fleisch, R. "Arch. ges. Physiol.," 1931, Vol 228, page 390.
Prosser, C. L. and others. "Radiology," 1947, Vol 49, page
    299.
Storey, R. H.; Wish, L.; Furth, J. "Proc. Soc. Exper. Biol.
    a. Med.," 1950, Vol 74, page 242.

The Course of the Exudative Phase of Inflammation in Irradiated Animals

E. R. Bagramyan

From the Radiation Laboratory (Head -- Docent D. E. Grodzenskiy) and the Department of Pathophysiology (Head -- Professor S. M. Leytes) of the All-Union Institute of Experimental Endocrinology

The problem of the course of the inflammatory process from the effect of X-rays on thebody attracted the attention of large groups of investigators as early as the first half of the 20th century. However, the majority of works devoted to this problem have dealt chiefly with the study of the morphological picture of experimental inflammation. It has been established by these investigations that in the irradiated organism the inflammatory reaction is depressed: the formation of giant cells, the emigration of white blood cells, etc. are inhibited (V. G. Garshin, V. V. Shikhodyrov and others). It seems interesting to elucidate the nature and the course of the exudative inflammation in radiation sickness, utilizing modern methods of investigation.

In the literature it has been repeatedly emphasized that under the influence of X-rays the permeability of the blood vessels is increased (S. B. Balmukhanov; P. N. Kiselev; B. N. Mogil'nitskiy and M. S. Brumshteyn; B. N. Mogil'nitskiy and others). In inflammation, as is well known, the blood vessel permeability is also increased. On the basis of this it may be considered that with the combined effect of X-rays and a stimulus producing inflammation the exudation should be increased compared with normal. On the other hand, taking into consideration the fact that exudation is a protective reaction of the body a lessening of it might be expected also as a result of a change in the reactivity of the body following the effect of ionizing radiation.

The present investigation had as its aim the elucidation of the following: 1) the influence of X-rays on the development of the exudative phase of inflammation; 2) the influence of irradiation on the protein composition of the exudate in comparison with the protein composition of blood serum of irradiated animals; 3) changes in the nature of the inflammatory reaction in irradiated animals when they are injected with ACTH.

The work was carried out on female rats. The inflammatory process was produced according to the method of Selye and consisted of the following. Twenty cubic centimeters of air were injected with a syringe into the subcutaneous

tissue of the interscapular area of the animals. The stimulus was injected into the air bubble formed -- 0.5 cubic centimeter of a 10 percent suspension of kaolin in peach oil. Thereby exudative inflammation developed. In one group of rats the inflammation was produced immediately after irradiation of the animals with X-rays in a sublethal dose (400 r). The second group of rats (non-irradiated) served as controls. The irradiation was carried out under the following conditions: voltage 180 kv, current 15 ma, skin-to-tube distance 40 centimeters, filters of one mm Al and 0.5 mm Cu. The dose rate was 35 r/min. The animals were weighed daily and their general condition was observed. In part of the rats on the fifth day of irradiation blood was taken from the caudal vein, and the white blood count was performed. In irradiated rats it amounted, on the average, to 2500 per cubic millimeter. In non-irradiated rats during the same period of inflammation the white blood count was 17,000-20,000 per cubic millimeter. On the 12th-14th day two cubic centimeters of the exudate was extracted from the inflammatory vesicle with a syringe. The ani als were killed. The exudate and blood serum was subjected to electrophoretic examination, and the concentration of total protein in them was determined refractometrically. We have described the method in detail in our previous work (1959).

Conditions of experiment	Day of inflammation						
	4 th	5 th	6 th	8 th	10 th	12 th	14 th
Non-irradiated							
Irradiated							
Irradiation + ACTH							
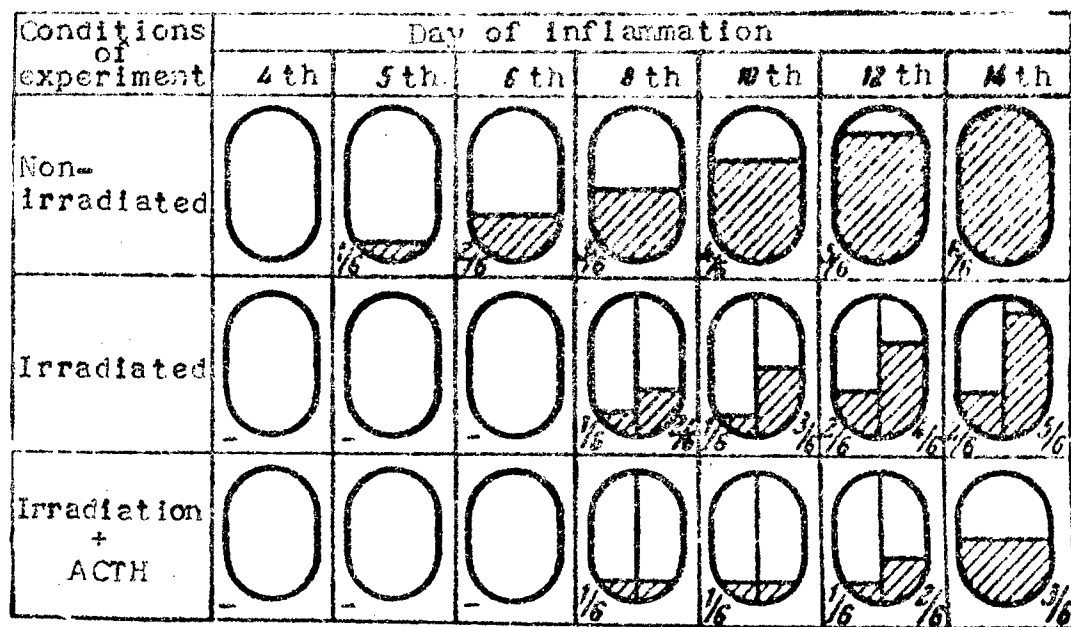
Dynamics of Accumulation of Exudate in the Inflammatory Vesicle (Explanation in Text).

At the same time, a study was made of the protein composition of the blood serum in intact and irradiated rats without inflammation.

On the Figure the dynamics of accumulation of the exudate in the inflammatory vesicle of non-irradiated and irradiated rats are shown. Frpm this Figure it is seen that the intensity of the accumulation of exudate in irradiated rats depends on the severity of the disease. In the event of severe radiation sickness, the index of which was the reduction in body weight, marked leukopenia which reached 1200 per cubic millimeter on the fourth day of inflammation, and other clinical signs of radiation sickness (diarrhea, adynamia, etc.) in our experiments, the exudation process was markedly inhibited (left half of the vesicle picture). The exudate level occupied no more than 1/6-1/3 of the vesicle cavity during the 12-14 days of inflammation. Usually, death of the animals occurred at this time. A small quantity of blood was not uncommonly found in the vesicle instead of exudate. In cases of a relatively mild radiation sickness the intensity of accumulation of the exudate was practically the same as that in non-irradiated animals (right half of the vesicle).

The exudate in the irradiated animals was hemorrhagic, not uncommonly of a dark brown color (hemolyzed). In control rats it has a bright yellow hue and contained an insignificant quantity of red blood cells. In its size the inflammatory vesicle was smaller in the majority of cases than in the non-irradiated animals. Its color was very much different from the color of the vesicle in non-irradiated animals and had a bluish-purple hue. Inhibition of the exudative process in irradiated rats was brought about probably by the marked areactivity of the animal organism as a result of severe dystrophies produced by the effect of ionizing radiation.

In the Table the results are presented of electrophoretic investigation of the protein composition of blood serum of rats and their inflammatory exudate.

In the literature there are numerous works in which a study has been made of the protein composition of blood serum of animals and people exposed to the effect of X-rays. It was shown in the early investigations (Davy; Herzfeld and Schinz; Gloor and Zuppinger; Mahnert; Wichels and Behrens) that under the influence of X-rays the concentration of total protein in the blood is reduced, the albumin/globulin ratio is decreased. Afterwards, by means of the electrophoretic method of investigation a detailed study was made of the changes in the composition of the protein fractions. A reduction in the albumin and γ-globulin fractions was found

and an increase in the α-globulin fraction (I. I. Ivanov and others; G. R. Shilin'sa; Höhne, Jaster and Künkel; Höhne, Künkel and Anger; Knipping and Kowitz; Muntz and Barron, Stender and Elbert; Westphal and others; Winkler and Paschke). Similar changes occurred as early as two or three days after the effect of X-rays. The intensity of these changes depended on the dose of irradiation. With large doses the changes occurred early and were more pronounced.

Protein Composition of Inflammatory Exudate and of Blood Serum of Rats in Percentages

Experimental Conditions	No of Rats	Serum				
		Albumin	Globulin			
			α_1	α_2	β	γ
Intact rats.....	25	50.7±0.8	10.8±0.3	8.8±0.3	17.2±0.4	12.5±0.4
Irradiated	25	43.8±1.2	13.3±0.5	10.8±0.4	19.8±0.6	12.3±0.9
Non-irradiated with inflammation.....	31	41.6±0.9	12.5±0.4	11.8±0.4	19.0±0.7	15.1±0.5
Irradiated with inflammation.....	25	37.9±1.2	15.9±0.5	13.8±0.8	20.4±0.7	12.0±0.5

We were interested in the protein composition of the blood serum of rats in the late periods of radiation sickness (14-16th day), whereas the majority of authors studied the protein composition of blood serum in the first week after irradiation. Therefore, we performed a special series of experiments in order to study the protein composition of blood serum of irradiated rats without inflammation. The irradiation was carried out under the conditions mentioned above. The results obtained on 25 animals are presented in the Table. Through a comparison of the protein composition of the blood serum of intact and irradiated rats a reduction in the albumin fraction and in the total protein concentration and an increase in the α and β-globulin fractions are distinctly seen in the latter. These data correspond to the data in the literature. The reduction in the albumin concentration was apparently produced by a reduction in the influx of them from the liver into the blood as the result of liver damage from radiation sickness and, therefore, a lessening of albu-

min synthesis.

In the development of the inflammatory process in the blood serum of irradiated and non-irradiated rats almost the same changes occur, namely, a reduction in the percentage of albumin concentration and an increase in the concentration of α and β-globulins. Similar changes in the relationship of protein fractions of the serum of non-irradiated rats have been described in the literature (B. S. Kasavina and V. S.

Protein, Grams %	Albumin	Globulin				Protein, Grams %
		α_1	α_2	β	γ	
5.32±0.07	-	-	-	-	-	-
5.02±0.09	-	-	-	-	-	-
5.08±0.06	46.4±0.8	10.7±0.3	9.4±0.3	23.2±0.7	10.3±0.6	2.8±0.05
4.91±0.14	40.8±1.4	13.7±0.6	11.1±0.6	27.3±1.3	7.1±0.4	2.8±0.1

(The "Exudate" heading spans the Albumin, Globulin, and second Protein columns.)

Gorkin; Longsworth and others, Schedlovsky and Scudder). In the irradiated animals these changes were more pronounced and depended on the severity of the radiation injury. A different reaction was observed with respect to the γ-globulins. While the concentration of γ-globulin in non-irradiated rats increased somewhat during the development of inflammation in irradiated rats it decreased. A tendency was noted toward a reduction in the percentage concentration of γ-globulin with an increase in the severity of radiation sickness. As is well known, γ-globulin carries the immunogenic properties of the body. An increase in their concentration in the serum of non-irradiated rats with inflammation should be regarded as a natural protective reaction of the body. A reduction in their concentration should be considered a decrease in this protective-adaptive reaction from the combined effect of X-rays and an inflammatory stimulus. In the inflammatory exudate of rats of both groups all the protein fractions were found which existed in serum. The percentage concentration

of albumin and β-globulin in the exudate of non-irradiated rats was higher than in the serum, and the percentage concentration of α_1-, α_2- and γ-globulin was less.

In the Table the average figures of the percentage concentration protein fractions are presented as they occur in inflammatory exudate of irradiated rats without taking into consideration the severity of the radiation sickness. Therefore, with statistical treatment the root mean square deviation (m) obtained was high. In the study of the Table the impression is created that the changes in the protein fractions of the exudate of irradiated rats are generally the same as in the non-irradiated rats. We analyzed the data obtained in accordance with the severity of the radiation injury. The indices of it were the following: 1) the nature of the exudate (the more the exudate was hemolyzed the more severe the radiation sickness); 2) the quantity of exudate (the more severely the exudation was inhibited the more severe the radiation sickness); 3) the degree of leukopenia; 4) other clinical signs of radiation sickness. Here, the changes in the protein fractions of the exudate were the following. In mild radiation sickness, as in non-irradiated rats with inflammation, the percentage concentration of albumin and particularly of β-globulin in the exudate was greater than in the serum, while the concentration of α- and γ-globulin was less. In radiation sickness of moderate severity the percentage concentration of albumin in the exudate and in the serum were almost the same. In severe radiation sickness the percentage concentration of albumins in the exudate was, on the average, the same in irradiated and non-irradiated rats.

According to our data (1959), ACTH in non-irradiated rats with experimental inflammation inhibits the process of exudation and prevents and increases outflow of albumin and γ-globulin into the exudate from the blood serum. It was interesting to clarify the influence of ACTH on the exudation process in irradiated animals so as to determine the inflammatory reaction of the irradiated organism to the hormonal effect. It was shown that the injection of long-acting ACTH into the irradiated animals in a dose of two units daily for 10-12 days delays the exudation process, just as in the non-irradiated animals, and in a number of cases stops it completely. (ACTH zinc phosphate, Kaunas Meat Combine Pharmaceutical Plant, series No 2). At the same time, of mortality rate of the animals increased considerably. Death occurred sooner than in the untreated animals. In the former death occurred on the 8th-10th day; in the latter, on the 13th-14th day after irradiation. This death apparently was associated with the activation of catabolic processes by ACTH, particu-

28

larly the catabolism of proteins (S. M. Leytes) and probably with a general reduction of the immunobiological reactivity of the body under the influence of ACTH.

Conclusions

1. The concentration of albumin is reduced and the concentration of α-globulin is increased in the serum of irradiated and non-irradiated rats with aseptic inflammation. In contrast to non-irradiated rats the concentration of γ-globulin is reduced in the irradiated rats.

2. In the inflammatory exudate of the irradiated rats the percentage concentration of β-globulin is markedly increased by comparison with the serum. Changes in the percentage concentration of γ-globulin and albumin is related to the severity of radiation sickness.

3. In irradiated rats, just as in the non-irradiated rats, ACTH inhibits the development of exudation in aseptic inflammation.

4. The injection of ACTH into irradiated animals with inflammation increases their mortality rate.

Bibliography

Bagramyan, E. R. Problems of Endocrinology and Hormone Therapy, 1959, No 2, pages 22-27.

Balmukhanov, S. B. Proceedings of the Central Scientific Research Institute of Roentgenology and Radiology. Moscow, 1954, page 5.

Garshin, V. G. "Vestn. rentgenol' i radiol." [Herald of Roentgenology and Radiology], 1938, Vol 19, page 355.

Ibid., Vol 20, page 292.

Ivanovii, Balabukh, V. S.; Romantsev, Ye. F. and others. Metabolism in Radiation Sickness. Moscow, 1956, page 97.

Kasavina, B. S.; Gorkin, V. Z. "Byull. eksper. biol. i med." [Bulletin of Experimental Biology and Medicine], 1954, Vol 38, No 12, page 38.

Kiselev, P. N. In the book: "Problems of Roentgenology and Radiology," Moscow, 1955, page 22.

Leytes, S. M. "Arkh. pat." [Archives of Pathology], 1956, Vol 18, No 6, page 45.

Mogil'nitskiy, B. N.; Brumshteyn, M. S. In the book: "Problems of Capillary Permeability in Pathology." Moscow, 1959, Vol 1, page 61.

Mogil'nitskiy, B. N.; Gol'dberg, A. F.; Shekhonin, V. P. In the book: "Problems of Roentgenology." Moscow, 1952, page 268.

Shilin'sh, G. R. The Effect of X-rays on the Total Quantity
    and the Protein Fractions of the Blood. Self-ref-
    erenced dissertation. Riga, 1952.
Shikhodyrov, V. V. Sectional Proceedings of the All-Union
    Conference on Medical Radiology. Experimental Radi-
    ology Section. Moscow, 1956, page 94.
Davy, L. "Am. J. Roentgenol.," 1931, Vol 25, page 255.
Herzfeld, E.; Schinz, H. R. "Strahlentherapie," 1923, Vol
    15, page 84.
Höhne, G.; Jaster, R.; Künkel, H. A. "Klin. Wschr.," 1952,
    No 30, page 952.
Höhne, G.; Künkel, H. A.; Anger, R., opus cit., 1955, Vol 33,
    page 284.
Gloor, W.; Zuppinger, A. "Strahlentherapie," 1931, Vol 40
    page 438.
Knipping, H. W.; Kowitz, H. L. "Fortschr. Röntgenstrahlen,"
    1924, Vol 31, page 660.
Longsworth, L. G.; Schedlovsky, T. and MacInnes, D. A. "J.
    Exper. Med.," 1930, Vol 70, page 399.
Mahnert, A. "Zischr. physiol. Chem.," 1920, Vol 110, page 1.
Muntz, J. A.; Barron, E. G.; Prosser, C. L. "Arch. Biochem.,"
    1949, Vol 23, page 434.
Schedlovsky, T.; Scudder, J. "J. Exper. Med.," 1942, Vol
    75, page 119.
Selye, H. J. "Am. Med. Assoc.," 1953, Vol 152, page 1207.
Stender, H. St.; Elbert, O. "Strahlentherapie," 1952, Vol
    89, page 275.
Westphal, U.; Priest, S. G.; Stets J. and others. "Am. J.
    Physiol.," 1953, Vol 175, page 424.
Wichels, P.; Behrens, A. "Zischr. ges. exper. Med.," 1927,
    Vol 56, page 387.
Winkler, C.; Paschke, G. "Radiation Res.," 1956, Vol 5,
    page 156.

Study of the Electrical Properties of the Blood by the Hydration Method After Radiation Injury

Yu. Yu. Voronoy, A. T. Stovbun, A. F. Kosenko

From the Ukrainian Scientific Research Institute of Nutrition and the Ukrainian Scientific Research Institute of Blood Transfusion

After radiation injury changes occur in the body of the animal in all organs and tissues, and the metabolism is changed. Particularly marked disturbances occur in the blood; its morphological composition is altered, as is also the quality of the protein. It is well known that in any transformations of a substance a tremendous part is played by electrical forces. The macromolecule of the protein consists of a polypeptide linkage to which side-groups are attached which possess hydrophobic and hydrophilic properties. In aqueous medium these groups react with one another and with the surrounding medium, as a result of which the protein molecule is turned into a globule; the hydrophobic groups are chiefly arranged inside of it; the hydrophilic groups, outside. The surface of the globule consists of polar groups ($COOH$ and NH_2), which have different charges. Such a molecule serves as a source of electrical forces determining the possibility of its reaction with other charged molecules or media possessing polar properties. Different influences on the body along with the change in its functional condition also change the structural characteristics of the protein molecule in a definite way.

V. K. Tkach suggested a method for studying the electrical properties of liquid biological media based on changes in the hydration kinetics of these media. When blood is dissolved in water a process of reaction of the polar groups of proteins and blood ions occurs with water. The products of hemolysis of erythrocytes and serum proteins which contain polar groups as well as free ions form molecular electric fields around themselves possessing a very high field intensity at short distances, which is adequate for attracting water molecules to them. Around each charged and polar particle there is created a hydration film. The polar molecules of water are bound by ions and polar groups of protein along the field of the given charged particle.

The following phenomena have been made the basis of the hydration method. When various charges and polar groups (blood dissolved in water) are placed in a certain alternating electromagnetic field, which changes at a definite frequency, the charges and polar particles make certain periodic

movements. The nature of these movements is determined by the properties of the particles themselves and those of the solvent (when the properties of the external electromagnetic field are kept constant). With the increase in the hydration film around the ions and polar groups their mobility is reduced and the electrical conductivity of the substance decreases, because the degree of orientation of the polar particles in the external field is reduced. Here, the polarizability of the water is reduced, which is expressed in a reduction in its specific inductive capacitance. Therefore, the method estimates the magnitude and the character of electrical properties of biological media by means of a study of the kinetics of the processes of hydration of the medium being studied when it is diluted in a polar solution. The index of the degree of hydration consists of the changes in the specific inductive capacitance of the combination being studied (in the given case blood + water).

The specific inductive capacitance of a substance is its capacity for increasing the capacitance of a condenser filled with the fluid being investigated to the capacitance of the same condenser in a vacuum. In studying the dynamics of organic matter a determination of the absolute values of the specific inductive capacitance is not necessary, because the relative values also give quite an accurate characterization of the process being studied.

The apparatus proposed by V. K. Tkach is constructed according to the resonance method in the high frequency area. A short wave oscillator producing waves of the order of 40 meters and stabilized by quartz is used. For the purpose of measuring the changing capacitance the method of electrical beats was selected. The capacitance meter was made in the form of a quartz test-tube, outside which brass rings were attached. A variable capacitance meter was hooked up into the oscillator circuit. For the purpose of compensating for the capacitance detuning a system of capacitors with worm gear was used, making it possible to establish the capacitance changes with an accuracy of up to 10^{-3} picofarads. The resonance point of the natural frequency quartz circuit is determined by the threshold of stoppage of oscillations. A 6-Ye-5 tube serves as an indicator. For the purpose of providing constancy in the feed voltage electronic stabilization is used. The resonance of a certain given circuit is determined, and the quartz test-tube containing the solution being investigated is connected up into this circuit. The resonance frequency of the electrical circuit depends on the capacitance of the circuit, which in its turn depends on the specific inductive capacitance of the solution in the test-tube.

At the present time, there are a few works devoted to the study of the specific inductive capacitance of serum or blood. Fürth established the fact that the specific inductive capacitance of serum is reduced during the inactivation process. In 1929, Ye. A. Sel'kov and I. Ye. Balygin observed the same phenomenon after inactivation of serum heated to +56° for a half-hour. In various diseases the reduction in the specific inductive capacitance of the serum was different. In 1947-1948, V. G. Chernov studied the specific inductive capacitance of erythrocytes and blood serum in horses in various pathological processes. Similar investigations were accomplished in 1950-1951 by V. K. Tkach and V. G. Chernov. In these works it was shown that the electrical properties of biological media change in a regular fashion. The magnitude of these changes can serve as a certain criterion contributing to the evaluation of various changes occurring in the body.

In connection with the fact that changes in the protein metabolism are observed in radiation injury, it seemed interesting to study the electrical properties of blood proteins of irradiated animals. In the present work a study was made of the dynamics of the dielectric properties of the blood of dogs irradiated with X-rays in different dosages.

The experiments were performed on five dogs. In all the animals a study was made of the dynamics of the dielectric properties of the blood for one month before the irradiation. For this purpose 0.6 millimeters of blood was taken from the vein of the front paw of the dog and diluted in 10 cubic centimeters of doubly distilled water. The solution was put into a quartz test-tube between plates of a capacitor, and the change in the capacitance was determined every 5 to 10 minutes for one to one and a half hours. The investigation was begun no later than two minutes after taking the blood once a day. The results obtained from investigating the capacitance of the condenser (which is equivalent to the specific inductive capacitance) was plotted on a graph, and a curve was obtained of the dielectric properties of the blood. After establishing the nature of the dynamics of the dielectric properties of the blood a single irradiation of the animals with X-rays in a dose of from 50 to 1000 r was performed. Immediately after irradiation the examination of the dynamics of the dielectric properties of the blood was continued.

Below, we are analyzing the results of the investigations performed. These were carried out on the dog Sharik which weighed 15 kilograms. Examination of the blood before irradiation showed that the nature of the change in the specific inductive capacitance was the same in all experi-

33

ments. Immediately after beginning theinvestigation the
capacitance of the condenser decreased, which correspon-
ded to the reduction in the specific inductive capacitance.
This decrease was observed for 15-20 minutes and reached a
certain minimum, after which the specific inductive capa-
citance did not change until the end of the experiment
(see Figure).

Time, min.
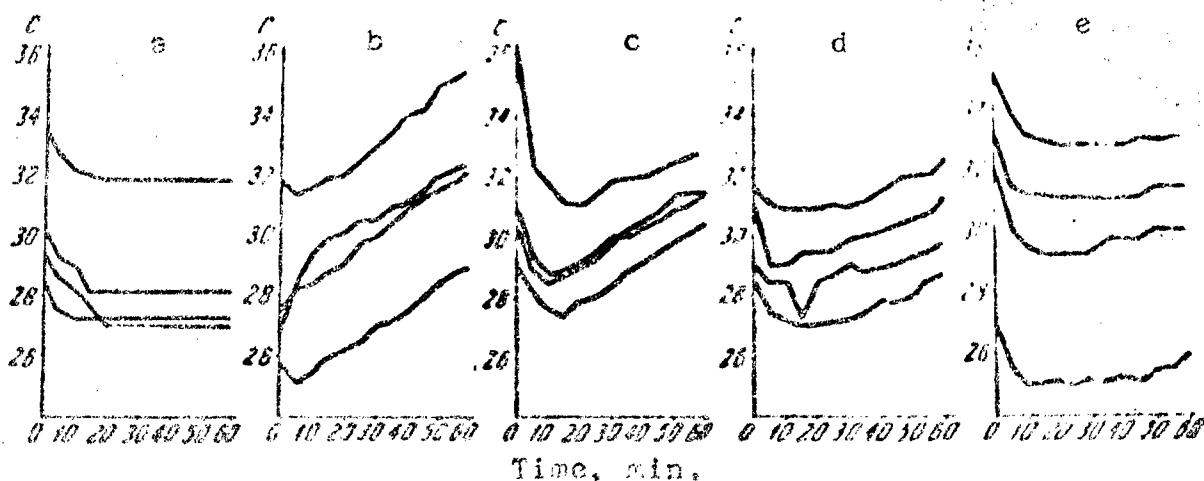
Change in the Dielectric Properties of the Blood in the Dog
Sharik after Irradiation with X-rays in a Dose of 500 and
600 r. The Condenser Capacitance (C) is Given in Relative
Units.

a -- before irradiation; b -- the first 10 days after ir-
radiation; c -- the second 10-day period; d -- the third
10-day period; e -- after 30 days.

Investigation of the blood before the irradiation was
carried out for a month, and then the dog was exposed to X-
irradiation. The irradiation was bipolar, with two appa-
ratuses: RUM-3 and RUM-11 under the following conditions:
voltage 180 kv, current 15 ma, skin-to-tube distance 50
centimeters in both apparatuses; filter 0.5 mm of Cu and 1
mm of Al. In the RUM-3 apparatus the dose rate was 24.1
r/min, the irradiation time was 20.8 minutes. In the RUM-11
apparatus the dose rate was 27.8 r/min, and the irradiation
time was 18 minutes. Therefore, the total dose of irradi-
ation was equal to 500 r.

An examination of the blood was begun on the day of
irradiation. It was established that during the first 10
days the nature of the change in the specific inductive
capacitance was radically changed. Immediately after be-
ginning the examination the capacitance of the condenser

34

was increased and continued to increase throughout the experiment. In certain cases during the first five minutes of the examination an insignificant decrease in the curve was observed (reduction in the capacitance, and then it increased inevitably the entire remaining time. These changes in the dielectric properties of the blood were observed as early as one hour after irradiation.

During the second 10-day period after irradiation the nature of the change in the dielectric properties of the blood was somewhat different. At the beginning of the investigations a pronounced reduction in the capacitance was noted which lasted up to 10-20 minutes, and then the capacitance increased for the entire time, reaching high values. In individual experiments the fall in the curve was of different degrees of intensity, and the rise was always marked and high. During the third 10-day period at the beginning of the experiment a fall in the curve was alsonoted for 5-20 minutes, and then the specific inductive capacitance increased for the entire time until the end of the experiment. However, this increase did not occur in such a sharp fashion as in the second 10-day period, was more prolonged and did not reach such high values. Afterwards, the degree of rise in the curve decreased, and the fall and increase in the specific inductive capacitance occurred more slowly. In various cases the curve of the change in dielectric properties of the blood was similar to the curve obtained before irradiation.

The general condition of Sharik during this period was the following. During the first 10 days after the irradiation the animal was sluggish; otherwise, its condition was almost the same as before irradiation. During the second 10-day period after irradiation the dog was inactive, refused food, had a bloody diarrhea, and cutaneous hemorrhages. In the third 10-day period the condition of the dog began to improve, and its appetite returned; the dog became more active, reacted normally to external stimuli. Thirty days after irradiation the condition of the animal was the same as before starting the experiments. The investigations showed that the change in the general condition of the animal after irradiation was in complete agreement with the change in the dielectric properties of the blood of the investigated animal.

Two months after the first irradiation, when the condition of the animal had become normal, the dog was irradiated again. The dose of irradiation was 600 r. Examination of the blood 23 minutes after the second irradiation showed that the specific inductive capacitance began to increase immediately, as had been observed also after the first irradiation. On subsequent days a slight decrease in the cap-

acitance was observed for a 10-minute period, and then a persistent increase in it which lasted until the end of the experiment. On subsequent days, before the death of the animal, a marked reduction in the capacitance was noted for 10-20 minutes and just as sharp a rise in it. On the sixth day after the second irradiation the dog died.

In the dog Sultan the changes in the dielectric properties of the blood before irradiation were of the same nature as in Sharik. The examination of the blood after irradiation of the animal in a dose of 600 r showed that as early as 18 minutes after irradiation a change is observed in the dielectric properties of the blood. The specific inductive capacitance increased sharply, without any initial decrease, throughout the entire experiment. Afterwards, a marked drop and then an abrupt rise in the specific inductive capacitance were noted. On subsequent days, before the death of the animal (a month after irradiation) the curve of change in the specific inductive capacitance did not show any falls either, but rather immediately after beginning the examination increased sharply. The general condition of the animal after irradiation was satisfactory, but beginning with the second week it began to deteriorate notably. There was shortness of breath, salivation, loss of appetite, bloody diarrhea, and muscle weakness. These signs increased and led to the death of the animal a month after irradiation.

Our investigations show that the change in the condition of the body after radiation injury is expressed in a change in the dynamics of the hydration processes of the blood proteins; this is expressed in a change in the specific inductive capacitance of the blood. The nature of the change in the curve of specific inductive capacitance of the blood of irradiated animals is directly related to the dose of irradiation, the severity of the general condition of the animal, and, to a certain degree, to the individual characteristics of each animal. The most pronounced changes were observed after a dosage of 500-600 r; they were expressed in an increase in the specific inductive capacitance of the blood of irradiated animals. The changes in the blood occurred as early as the first hour after irradiation, which makes it possible to utilize this method for the early diagnosis of radiation injury.

The nature of the change in the dielectric properties of the blood makes it possible to predict the course of the pathological process. The study of the dielectric properties of the blood can serve as one of the auxiliary methods in diagnosing and studying the nature of the course of radiation sickness.

Conclusions

1. The change in specific inductive capacitance of the blood in all animals before irradiation is of the same character.

2. Irradiation of the animals with X-rays in different doses produces a change in the dielectric properties of the blood which is expressed in an increase in the specific inductive capacitance, the nature of which depends on the dose of radiation and the development of the pathological process.

3. After irradiation with a dose of 500-600 r the changes in the specific inductive capacitance of the blood are observed as early as the first hour after irradiation.

Bibliography

Sel'kov, Ye. A.; Balygin, I. Ye. In the book: "Collection of Scientific Works Devoted to N. N. Petrov." Leningrad, 1939, page 26.

Tkach, V. K.; Chernov, V. G. In the book: "Collection of Works of the Khar'kov Veterinary Institute," 1952, Vol 21, page 135.

Obtaining Diphtheria Toxin in Media Sterilized with Gamma-Rays

D. R. Kaulen

From the Department of Radiation Microbiology and Immunology
(Head -- Professor V. L. Troitskiy) of the Institute of Epi-
demiology and Microbiology imeni Gamaleya of the Academy of
Medical Sciences USSR

In the literature available to us we have not been
able to find any reports about the sterilization of nutrient
media with ionizing radiation; nevertheless, certain works
should be discussed briefly which are indirectly related to
this problem.

I. P. Mishchenko and M. M. Fomenko, in studying the
influence of X-irradiation (doses of 20-100 percent NED) on
a 24-hour bouillon culture of staphylococcus or colon bacil-
lus, noted a definite depression in the enzymatic activity.
The authors came to the conclusion that the medium is
changed in the direction of a greater or lesser decomposi-
tion of protein molecules under the influence of X-rays. With-
out analyzing in detail what was the object of the direct
effect of the X-rays (the microbe or the medium), the authors
drew the a priori conclusion that irradiation of the medium
will depress the formation of toxin by bacteria, because
their enzymatic activity is depressed. In certain works it
has been shown that irradiation of the media in a dose of
600 r and even in a dose of 81,800 r does not interfere with
the growth of leishmanias (L. F. Burova, S. A. Molchanova)
and the growth of diphtheria bacilli on simple bouillon or
on Martin's bouillon (D. S. Kostyrko). On the other hand,
S. G. Rybakova showed that irradiation of wort with radium
emanations (26-19 µC for 24 hours) or wort-agar (78 µC for
24 hours) leads to an inhibition of growth of S. cerevisiae
on them but not to a complete suppression of them. She
showed also that extracts from irradiated aqueous agar stimu-
late the growth of yeasts.

O. G. Alekseyeva and M. P. Domshlak reported that ir-
radiation of agar in doses above 30,000 r produce a change
in pH in an alkaline direction which influences the growth
of bacteria. In doses of 30,000-150,000 r an increase in
growth is observed in 45-50 percent of the cases; with doses
of 150,000 to 2,000,000 r, either a depression of growth or
no change in the growth. It has been noted that as a result
of irradiation the medium acquires bactericidal properties,
which after a dose of 90,000 r are maintained maximally for
67 hours. The colon bacillus is more sensitive to these
changes than the staphylococcus.

As seen from the reports presented the results of the

works are contradictory.

It should be emphasized that all the investigators mentioned above did not pursue the aim of sterilizing the nutrient media; they used relatively small doses of radiation and, which is important from our point of view, irradiated media which were already sterilized by autoclaving. Nevertheless, the use of γ-rays in the production of bacteriological preparations and, particularly, in the sterilization of nutrient media opens up broad perspectives before research workers (V. L. Troitskiy and others).

The nutrient medium (Poppet's bouillon, Martin's bouillon, tryptic digest medium) was poured out into small (200 cubic centimeter) matrasses in quantities of 50 cubic centimeters and irradiated with γ-rays in doses of 600,000, 1,000,000 and 1,500,000 r. The irradiation was carried out on an EGO-2 apparatus with radioactive Co^{60}, with a total dose rate of 5,000 C. The dose rate was 600 r per minute. In each experiment there were several groups: one was autoclaved and several were sterilized by irradiation.

In each group there were four or five matrasses. In certain experiments the medium was autoclaved and then irradiated. The culture was made five or six days after sterilization. For culture purposes use was made of a 24-hour growth of the standard strain of PW-8 diphtheria culture. Before the seeding 0.5 cubic centimeter of 40 percent glucose solution was added to the medium. Cultivation was carried out for six to seven days, after which the diphtheria toxin was filtered off through a paper filter. In order to avoid accidental variations in the values of the toxin titer, a mixed toxin was prepared from the contents of all the matrasses of each group. The strength of the toxin was determined according to the flocculation reaction and according to the Ln (minimum quantity of toxin which causes necrosis after intracutaneous injection) in guinea pigs.

In certain experiments toxoid was prepared, for which purpose formalin was added to the toxin obtained up to the concentration of 0.4 percent formaldehyde, and the mixture was put into an incubator at 40° for 30 days. After this, the preparation was checked for toxicity and immunogenic properties.

In addition a study was made of the possible changes in the biochemical composition of the medium under the influence of irradiation. The total and protein nitrogen (Kjeldahl method), the amine nitrogen (method of Sörensen-Gavrilov), the pH (according to the Michaelis method), tryptophane (according to the Peshkov method) and the concentration of amino acids and polypeptides (according to the method of Willstädter and Waldschmidt-Leitz) were determined. In

those cases where the cultivation of the diphtheria culture was carried out, these biochemical indices were determined also in the toxin obtained.

In all experiments without exception where the radiation doses mentioned above were used a complete sterility of the medium was achieved.

In Certain cases the medium sterilized by irradiation was considerably clearer than the autoclaved medium.

The growth of the PW-8 culture on media sterilized by irradiation was no different from that on autoclaved media. On the second day, a surface film was formed which became thicker and settled to the bottom. The bouillon remained transparent.

In 10 experiments the strength of the toxin was determined by flocculation and by the flocculation time (Table 1). From Table 1 it is seen that in the absolute majority of cases the toxin titer obtained in the medium sterilized by irradiation was either the same as the titer of toxin obtained in the autoclaved medium or exceeded it.

Table 1

Formation of Diphtheria Toxin on Irradiated Media

Medium	Titer According to Flocculation					Time of Flocculation in Minutes				
	Not Irradiated	600,000 r	1,000,000 r	1,500,000 r	2,500,000 r	Not Irradiated	600,000 r	1,000,000 r	1,500,000 r	2,500,000 r
Poppet's bouillon...	60	66	–	60	62	30	30	–	35	25
Poppet's bouillon...	52	68	68	68	–	25	20	20	20	–
Poppet's bouillon...	28	64	–	40	–	45	15	–	35	–
Poppet's bouillon...	38	62	50	46	–	35	15	25	25	–
Poppet's bouillon...	46	62	50	52	–	80	70	80	80	–
Poppet's bouillon...	52	–	–	34						
Tryptic digest...	68	76	–	78	–					
Martin's bouillon...	66	74	–	66	–					
Martin's bouillon...	36	40	–	36	–					
Poppet's bouillon...	64	74	–	70	–	20	15	–	20	–

Table 2

Certain Biochemical Indices of Irradiated Nutrient Media

Medium	Cultivation	Irradiation	pH	Total N, mg%	Protein N, mg%	Amine N, mg%	Trypto-phane, mg%	Amino Acids, mg%	Poly-peptides, mg%
Tryptic digest	Before culture	Not irradiated.	8.0	359.8	—	95.2	66	34.86	78.68
		600,000 r.	8.0	357.6	—	93.8	66	34.86	78.68
		1,500,000 r.	7.9	357.0	—	93.1	66	34.86	78.68
	After culture	Not irradiated.	8.9	315.0	—	110.6	—	62.3	68.32
		600,000 r.	8.75	294.0	—	116.0	—	23.94	116.06
		1,500,000 r.	8.7	298.2	—	110.6	—	54.88	74.48
Martin's bouillon	Before culture	Not irradiated.	7.7	446.6	49.28	94.5	—	43.96	80.08
		600,000 r.	7.7	434.0	28.0	94.5	—	41.72	81.20
		1,500,000 r.	7.7	438.2	40.27	95.9	—	41.72	81.20
	After culture	Not irradiated.	8.4	405.8	5.46	119.0	—	49.56	83.86
		600,000 r.	8.4	383.6	8.54	119.0	—	58.66	79.94
		1,500,000 r.	8.4	401.4	8.4	117.6	—	53.20	85.40
Martin's bouillon	Before culture	Not irradiated.	7.8	443.8	5.18	91.07	—	35.14	72.94
		600,000 r.	7.8	420.0	4.34	89.74	—	42.14	70.14
		1,500,000 r.	7.7	407.3	4.34	89.74	—	29.26	67.06
	After culture	Not irradiated.	8.6	399.0	9.24	117.6	—	43.96	93.38
		600,000 r.	8.6	403.2	6.16	125.3	—	58.66	86.66
		1,500,000 r.	8.6	346.8	7.84	119.0	—	51.24	88.76
Poppet's bouillon	Before culture	Not irradiated.	8.0	364.0	10.22	114.8	122	48.58	78.82
		600,000 r.	8.0	355.6	4.76	113.4	110	44.66	82.74
		1,500,000 r.	8.0	352.8	3.50	106.4	96	44.66	82.74
	After culture	Not irradiated.	8.8	324.8	5.32	126.7	—	35.0	99.40
		600,000 r.	8.8	294.0	4.76	144.9	—	35.0	112.20
		1,500,000 r.	8.8	291.2	4.04	158.2	—	31.08	117.30
Martin's bouillon	Not performed	Not irradiated.	7.6	473.0	12.4	91.0	—	30.58	66.08
		600,000 r.	7.5	476.0	10.9	97.0	—	—	—
		1,500,000 r.	7.5	465.0	12.4	98.0	—	34.86	68.18

[continued]

41

Table 2 [continued]

Medium	Cultivation	Irradiation	pH	Total N, mg%	Protein N, mg%	Amine N, mg%	Tryptophane, mg%	Amino Acids, mg%	Poly-peptides, mg%
Martin's bouillon	Not performed	Not irradiated.	7.9	453.6	6.58	94.5	—	27.04	75.52
		600,000 r.	7.9	456.4	—	94.5	—	27.04	75.52
		1,500,000 r.	7.9	455.0	6.58	94.5	—	27.04	75.52
Martin's bouillon	Not performed	Not irradiated.	7.6	373.8	7.56	88.2	64	33.04	79.24
		600,000 r.	7.6	364.0	8.9	88.2	64	29.36	78.54
		1,500,000 r.	7.6	375.2	6.36	88.2	64	29.26	75.04
Tryptic digest	Not performed	Not irradiated.	8.0	352.8	3.36	94.5	73	29.26	73.64
		1,500,000 r.	8.0	354.2	3.36	88.2	73	26.12	74.76

The determination of the strength of the diphtheria toxins according to the Ln on guinea pigs in general gave results similar to the results of the flocculation reaction.

In those cases where cultivation was carried out on autoclaved media and then irradiated media the formation of toxin was considerably less. The latter was confirmed by the flocculation reaction, the flocculation time and the Ln.

In two experiments with three filtrates of the medium sterilized by autoclaving and irradiation with a dose of 1,500,000 r the PW-8 culture gave no flocculation reaction after being cultivated in it. Nevertheless, there was toxin in it, judging by the Ln, although in considerably smaller quantities.

The results of biochemical tests (Table 2) show that in liquid nutrient media no essential changes occur. The pH of the medium remains unchanged. The concentration of total and amine nitrogen in the medium is unchanged.

Considerable variations in the concentration of protein nitrogen (which occurred in certain experiments) apparently must be attributed to the method of determination, wherein losses are possible during the separation of the protein by means of centrifugation. In certain cases a change occurs in the quantity of amino acids and polypeptides; however, these changes are of a chance nature and cannot be systematized.

A study of the properties of the toxoids gave the following results.

Toxoid obtained from toxin from the medium sterilized by irradiation (toxoid I) did not cause the death of mice after intraperitoneal injection in a volume of 0.5-0.25-0.125-0.0625 cubic centimeter, nor did toxoid obtained from the toxin from the autoclaved medium (toxoid II). The toxoids which we obtained were not toxic for guinea pigs after subcutaneous injection in a volume of five cubic centimeters (observation time 30 days). In one of the experiments the toxoid obtained showed a flocculation of 36 LF (flocculation units) just as did the original toxin; here, the time of flocculation of the toxoid I was less than of toxoid II. These preparations were injected into guinea pigs subcutaneously in doses of five cubic centimeters. After 30 days the resistance of the animals was checked by 30 MLD of diphtheria toxin. It was shown that toxoid I not only did not lag behind the control preparation in its immunogenic properties but even exceeded the latter somewhat.

Whereas in immunization with the control preparation necrosis of the skin occurred in four guinea pigs out of 10 after the injection of 30 MLD of the toxin, in immunization with the experimental preparation it occurred in only two. In another experiment (Table 3) it was also seen that toxoid I did not lag behind toxoid II in its immunogenic and antigenic properties and in the titer of antitoxin formed it even exceeded the latter somewhat. Blood for the determination of the toxoid was taken from five guinea pigs of each group 28 days after immunization, a mixed serum was made and titrated according to the intracutaneous method on rabbits.

Table 3

Immunogenic and Antigenic Properties of Toxoids

	Guinea Pigs Used	Subcutaneous Immunization	Interval (Days)	MLD of Toxin Injected	No Died	No Survived	Toxoid Titer in Antitoxic Units
Toxoid I	14	5 cc	30	30	9	5	0.002
Toxoid II	15	5 cc	30	30	13	2	< 0.0005
Control	3	—	—	1	3	0	—

The experimental data obtained indicate the possibil-

ity of radiation sterilization of liquid nutrient media, which is confirmed by the following data. As a result of irradiation no essential changes in the physico-chemical characteristics occur in the nutrient media. Certain variations in the quantity of total and protein nitrogen are within the limits of experimental error.

The latter was computed by the method of root mean square errors on the basis of 30 determinations and was equal to ±5.6 milligrams percent of nitrogen. The concentration of amine nitrogen and tryptophane were practically unchanged.

In contrast to the data of O. G. Alekseyeva and M. P. Domshlak we did not notice any changes in the pH of the medium under the influence of irradiation. After cultivation, on the other hand, a considerable change in the pH of the medium occurs in an alkaline direction. The quantity of total nitrogen is reduced, and the quantity of amine nitrogen is increased. These changes, to a certain degree, correspond to the yield of diphtheria toxin.

The formation of diphtheria toxin on media irradiated with a dose of 1,500,000 r is the same as for non-irradiated media sterilized by autoclaving. With irradiation of the medium with 600,000 r the formation of toxin is even improved. This situation deserves attention, since irradiation in a dose of 600,000 r is sufficient to destroy the vegetative forms of bacteria. Finally, toxoid prepared from toxin obtained from irradiated medium did not have any toxic side-effects and was not inferior to control preparations in its immunogenic and antigenic properties. On the other hand, the combination of autoclaving, and irradiation impoverishes the properties of the nutrient medium, and the formation of diphtheria toxin on it is much less. The cause of this is not as yet clear.

Our data have not confirmed I. P. Mishchenko's and M. M. Fomenko's suggestions that it might be possible to depress the formation of toxin on irradiated media, although the species of bacteria and the medium utilized and the treatment of it may have considerable significance. Actually, in those cases where the bouillon utilized was autoclaved and then irradiated with a sterilizing dose of γ-rays the formation of toxin on it was much less. However, it should be noted that in medium treated in this way the diphtheria culture produced a good growth in the form of a solid film which settled to the bottom.

The biochemical investigations available to us did not give us an answer to the question of why a better production of diphtheria toxin occurs on media irradiated with a dose of 600,000 r. Apparently more thorough and precise analyses of the amino acid composition of the irradiated

media, a study of the effect of γ-rays on vitamins, etc. are necessary. Despite certain elements which are not clear, "cold" sterilization of liquid nutrient media is possible and even expedient. The latter consideration may be illustrated by the following example. Martin's nutrient bouillon is sterilized by autoclaving fractionally for three days. For the purpose of radiation sterilization a single irradiation for 16 1>2 hours (600,000 r) or 42 hours (1,500,000 r) is adequate using an experimental EGO-2 radiator. However, at the present time there is every possibility of increasing the dose rate of the radiators and, therefore, reducing the time of the treatment.

Conclusions

1. The biochemical composition of liquid nutrient media sterilized by γ-rays in doses of 600,000 r and 1,500,000 r is not subject to any essential changes by comparison with the autoclaved media.
2. The titer of diphtheria toxin (according to the Lf and Ln) obtained in irradiated media is not inferior to that obtained in autoclaved media and in certain cases (irradiation of the medium with 600,000 r) even exceeds it.
3. The toxoids obtained are not inferior to the control preparations in their immunogenic and antigenic properties.
4. The experimental data indicate the possibility of "cold" sterilization of liquid nutrient media utilized for obtaining diphtheria toxins by γ-rays.

Bibliography

Alekseyeva, O. G.; Domshlak, M. P. "Byull. rad. med." [Bulletin of Radiological Medicine," 1954, No 1, page 75.
Burova, L. F.; Molchanova, S. A. "Med. mysl' Uzbekistana" [Medical Thought of Uzbekistan], 1930, No 7-8, page 47.
Mishchenko, I. P.; Fomenko, M. M. In the book: "Experimental and Clinical Roentgenology." Khar'kov, 1928, Collection 2, page 50.
Rybakova, S. G. Works of the Central Scientific Research Institute of Roentgenology and Radiology. Moscow, 1941, Vol 5, page 217.
Troitskiy, V. L. "Med. radiologiya" [Medical Radiology], 1957, Vol 2, No 5, page 80.
Bellamy, W. D.; Goldblith, S. A.; Colovos, G. C. and others. "Bact. Rev.," 1955, Vol 19, page 226.

Bierman, G. W.; Proctor, B. E.; Goldblith, S. A. "J. Dairy
    Sc.," 1956, Vol 39, page 379.
Goldblith, S. A.; Proctor, B. E. Ibid, page 374.
Schultz, H. W.; Cain, R. F.; Nordon, H. C. and others.
    "Food Technol.," 1956, Vol 10, page 233.

Characteristics of the Distribution and Excretion of Polonium in Animals with the Use of Unithiol

Ye. V. Erleksova

The aim of this work was an elucidation of the effect of unithiol [HS-CH$_2$-(SH) · CH$_2$SO$_3$Na · H$_2$O] on the process of distribution and excretion of polonium from the body and the nature of changes in the animal organs under the conditions where this preparation is used.

There are no special works devoted to the description of the pathomorphology and investigation (by means of the photo method) of the distribution and excretion of polonium from the bodies of animals after the effect of unithiol.

Unithiol is a sulfo acid derivative of 2, 3-dimercaptopropanol, which is readily soluble in water and which gives a reaction with heavy metals forming mercaptides which are soluble in water. It was used for accelerating the excretion of polonium (which belongs to the radioactive heavy metal group) from the bodies of white rats. Here, it was taken into consideration that water-soluble mercaptoid-like complexes formed in the body with polonium through the effect of unithiol will be excreted freely in the urine by the kidneys and partly in the stool.

The work was carried out on 110 white male middle-aged rats weighing from 150 to 180 grams (of these 50 were experimental, 50 control, and 10 rats were injected with unithiol). A solution of polonium (0.1 mC/kg) in mannitol was injected once under the skin. The aqueous solution of unithiol was also injected subcutaneously twice a day, using it in the following way; in the first three days it was given according to the calculation of 100 milligrams/kilogram; afterwards, the dose was reduced to 50 milligrams/kilogram. On the first day of injection the polonium preparation was injected one and five-six hours before the experiment, and for the first six days it was injected daily; after that, there was a three-day interruption with a subsequent injection of it for four days, after which it again was interrupted for three days, and then injected again, etc.

According to the data of the investigation of the activity state of the urine and stool it was shown that the rate of excretion of polonium in the urine and, to a lesser degree, in the stool was increased by three times compared with the control under the influence of unithiol (M. A. Zaytseva). Control animals not treated with unithiol all died on the 11th-13th-20th day after the injection of this element. The length of life of the treated rats was in-

creased; death of them occurred on the 33rd-115th day after the injection of polonium; part of them were killed at the time the control animals died for the purpose of gaining knowledge of the effect of unithiol on the processes of distribution and excretion of polonium from the body.

For the purpose of studying the nature of the changes in the organs of both control and experimental white rats a morphological investigation was performed. Pieces of the organs were fixed in 10-percent neutral formalin. The sections were stained by the usual histological methods. For the purpose of obtaining autoradiographs movie film was used (with a sensitivity of 90 according to the GOST [All-Union State Standard]). Sections from unfixed organs with a thickness of 30 μ were prepared with a freezing microtome for the purpose of obtaining autohistoradiographs thick-layered NIKFI [Motion Picture and Photography Scientific Research Institute] plates of the A_2 type having a thickness of the emulsion layer of 50 μ were used.

For the purpose of obtaining fine sections (5 μ) with this method of investigation the material was embedded in paraffin. All the preparations were exposed under the same conditions for three days.

In comparing the data of investigation obtained from the autoradiographs and the histoautoradiographs of the control and experimental animals it was shown that a smaller quantity of polonium was contained in the organs of the experimental white rats; this was particularly distinctly expressed in the hematopoietic organs: in the spleen and in the lymph nodes of the entire body. As far as the organs of the excretory system are concerned -- the kidneys -- a large quantity of polonium was contained in them, on the other hand, in the experimental rats than in the control animals. This element was concentrated chiefly at the border between the cortical and medullary layer (Fig. 1).

Similar data were obtained also in the investigation of histoautoradiographs of these organs. In addition, there was no greater number of macrophages and therefore "stars," that is, accumulations of radioactive element in these cells, in the experimental rats than in the controls.

At autopsy of the cadavers of the control white rats which died 11-13-20 days after the subcutaneous injection of polonium (0.1 mC/kg), a distinctly expressed picture of acute radiation sickness was observed: signs of hemorrhage in the lungs, mucous membranes of the gastrointestinal tract and hematopoietic organs (spleen, lymph nodes and bone marrow), aplastic anemia and dystrophy of the internal organs.

In the lungs the hemorrhages were of a focal character and were located both under the pleura and in the center

of the parenchyma. In addition, marginal emphysema and focal edema of the lungs were found. In one rat a hemorrhagic pneumonia was noted. The mucous membranes of the gastrointestinal tract were slightly edematous in places, chiefly in the small intestine, and focal hemorrhages of different sizes were observed. The hematopoietic organs were markedly congested: the spleen was reduced in size; the lymph nodes of the entire body, on the other hand, were enlarged, succulent and with signs of hemorrhage. There were no apparent changes in the glands of internal secretion (pancreas, thyroid gland, sexual organs -- testicles and adrenals).
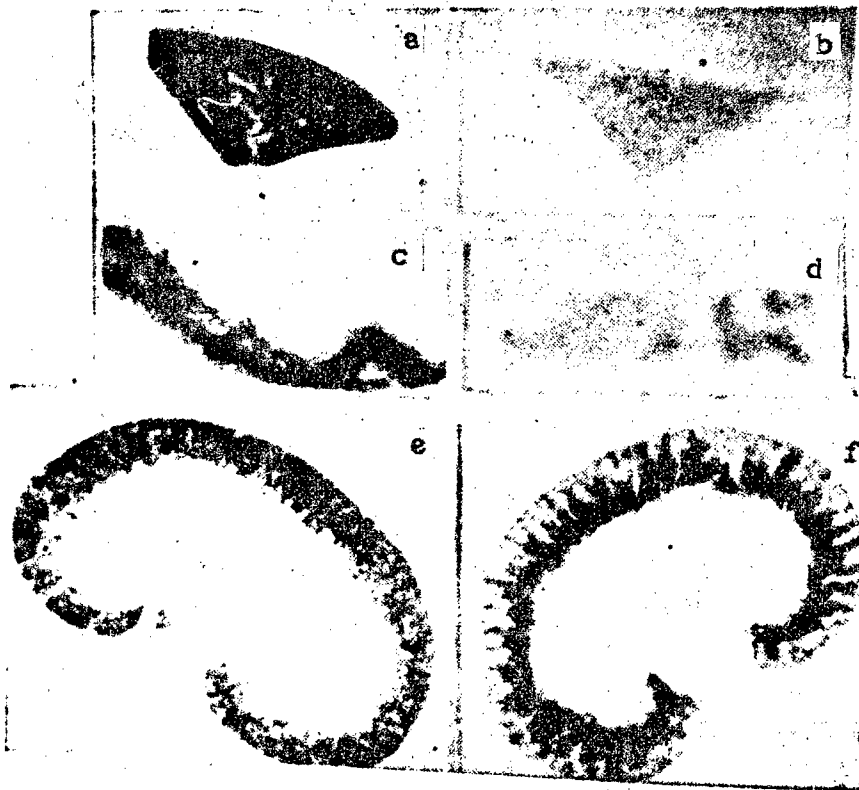
Fig. 1. Autoradiographs of Organs of Control Rat (on the Left) and Experimental Rat (on the Right) on the 13th Day After Injection of Po^{210} Calculating 0.1 mC/kg. Exposure Time Three Days.

a, b -- spleen; c, d -- mesenteric lymph node; , f -- kidney.

At autopsy of the cadavers of the experimental white rats killed at the time the control animals died (11th-13th-20th day) a somewhat different macroscopic picture was observed in the organs and tissues, namely: first, not so

many hemorrhages were found in the lungs and in the mucosae of the gastrointestinal tract (there were practically no signs of hemorrhage); secondly, the volume of hematopoietic organs, spleen as well as lymph nodes, was close to being normal. The lymph nodes were somewhat congested and slightly succulent.

As far as the other organs are concerned, in the experimental rats they were markedly congested by comparison with the controls, chiefly the liver and kidney. In addition, the parenchyma was of a flabbier consistency.

In animals which lived longer after the injection of the polonium (102-115 days), the macroscopic picture was similar to the pathological picture observed in chronic radiation sickness as a result of the effect of moderate doses of polonium (0.02-0.003 mC/kg), where exhaustion, indolent skin ulcers (without apparent development of granulation tissue) were found also at the site of injection of polonium, atrophy of the testicles and a reduction in their volume because of an atrophy of the cortical layer. The hematopoietic organs were somewhat reduced in size (spleen and lymph nodes). In the parenchymatous organs distinct dystrophic changes and congestion were noted (in the liver, heart, kidneys, etc.).

In rats which were given only unithiol and which were killed on the 38th-40th day after the experiment was begun a marked congestion of all organs and systems was found, chiefly of the liver and kidneys, which was not observed in the killed control rats.

The data of histological investigation of the organs obtained confirmed the differences which we found in autopsy of the animal cadavers in connection with the degree of severity of the developing changes in the organs of control and experimental animals, particularly in the hematopoietic organs and kidneys.

In the control rats, which died on the 11th-12th-20th day after beginning the experiment, a cloudy swelling of the endothelium and blood vessel walls with signs of plasmorrhage and hemorrhage was noted.

The capillaries of the interalveolar septa were markedly dilated and overfilled with blood. In the lumina of the alveoli there was a serous fluid and desquamated alveolar epithelial cells. In places, the alveoli were completely filled with erythrocytes. The epithelium of the bronchi did not show any particular changes. In one rat a focal necrotic bronchial pneumonia of hemorrhagic nature was found without any cellular leukocytic infiltration.

In the heart an irregular staining of various muscle fibers was found. The mucous membranes and submucosae of

the gastrointestinal were edematous. In them foci of hemorrhages were found. In places, the epithelium of the mucous membranes had desquamated. In the liver there was edema of the stroma and a marked dilatation of the lumina of the intertrabecular capillaries, which were overfilled with erythrocytes. In places in them macrophages were encountered which contained hemosiderin and polonium. Cloudy swelling of the Kupffer cells, of the cytoplasm of the liver cells and epithelium of the biliary passages was found. The kidneys were congested; the epithelium of the convoluted tubules were in a state of slight cloudy swelling. The other parts of the tubular apparatus as well as the glomeruli were practically within normal limits. In the hematopoietic organs (in the spleen and lymph nodes) there were no lymphoid elements in the follicles. In places karyorrhexis of these cells was observed. Against this background the reticular stroma stood out in relief; the cells of it did not show any apparent changes. A considerable number of plasma cells was present, and there was a particularly large number of them in the medullary cords of the lymph nodes. In the dilated sinuses of the lymph nodes there was a large number of macrophages and erythrocytes. The macrophages were filled with erythrocyte fragments and polonium.

With respect to the organs of the endocrine system it was possible to find changes in the suprarenal glands expressed in cloudy swelling of the cytoplasm of the cells of the cortical layer with an absence of lipoid in them. In the ovaries there was frequently noted a pronounced disarrangement of the germinal epithelial cells.

At the site of injection of the polonium in the subcutaneous fat tissue there was a focal hemorrhage, a large number of histiocytes containing polonium, and tissue necrosis, necrosis of the blood vessel walls and underlying muscles with an absence of cellular and leukocytic infiltration.

The data of the histological examination of experimental animals showed that the dystrophic changes of the organs (heart, liver, lungs, etc.) were similar to changes in the organs of control rats with the exception of the kidney, an organ which belongs to the excretory system. The most serious changes in the kidneys by comparison with the control animals occurred as the result of an acceleration of excretion of polonium in large quantities through this organ as the result of the effect of unithiol. These changes were expressed as early as the 13th day of the experiment in a markedly cloudy swelling of the epithelium of the convoluted tubules, in places with signs of karyorrhexis and marked congestion of the organ, chiefly at the boundary be-

tween the cortical and medullary layers where the polonium
was retained.
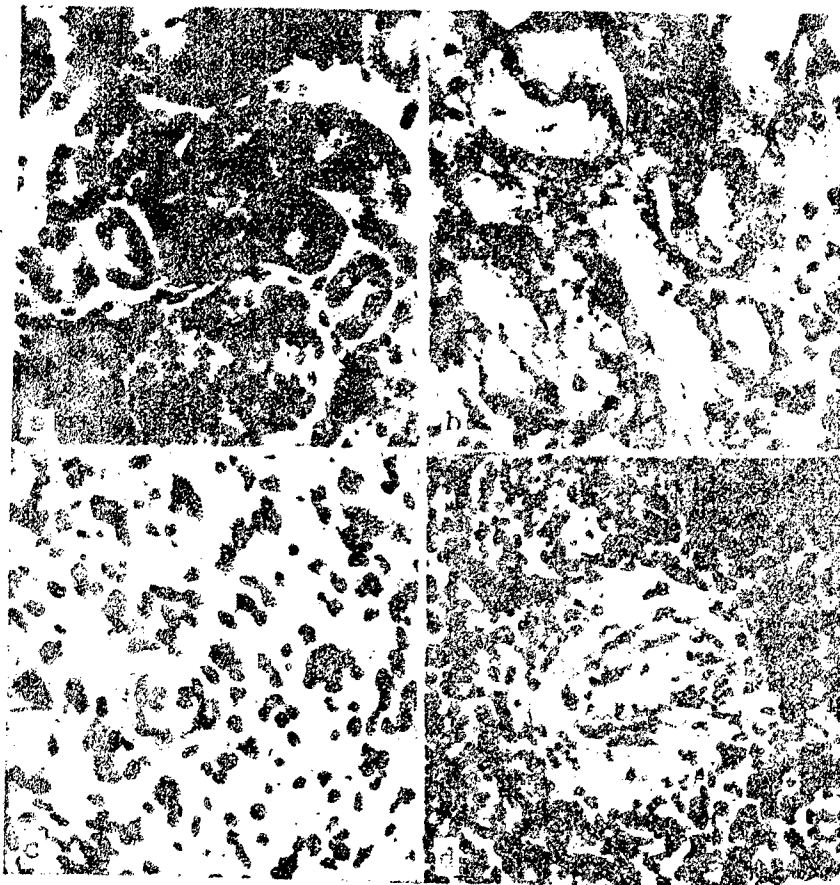
Fig. 2. Microphotograph of Organs of Control Rat (on the
Right) and Experimental Rat (on the Left) after
the Injection of Po^{210} Calculating 0.1 mC/kg.

a -- kidney; slight cloudy swelling of epithelium of convo-
luted tubules; b -- kidney; more pronounced cloudy swelling
of epithelium of convoluted tubules at the same time of
life as in the control animal; c -- spleen; disappearance
of lymphocytes from follicles, and cloudy swelling of the
wall of the central artery; d -- spleen; well-preserved
lymphoid follicles.

In the spleen and lymph nodes there were no severe
destructive changes observed after the injection of uni-
thiol. The number of lymphoid elements in the follicles
was largely preserved by comparison with that in the con-
trol rats. The follicles were clearly seen. Such a con-
dition of the hematopoietic organs lasted until the 35th

day. Afterwards, the number of lymphocytes began to drop
noticeably, and plasma cells appeared (Fig. 2). In the ex-
perimental rats which lived for a longer time and which were
fiven unithiol (from 33 and up to 115 days) dystrophic
changes were seen in the entire field on histological exam-
ination of the kidneys. Residues of the convoluted tubules
were encountered consisting of one to two epithelial cells
with large hyperchromatic nuclei. In the lumina of many
tubules there were hyaline casts, and in the excretory
tubules desquamated necrotic epithelial cells were seen.
The glomeruli were relatively little changed. Their capsules
were somewhat thickened, and the lumina of the blood vessel
network were dilated and contained erythrocytes. The kidney
stroma was somewhat thickened, homogenized and markedly con-
gested, chiefly at the boundary between the cortical and
medullary layer. These changes were most distinctly expres-
sed on the 102nd-115th day after the injection of polonium,
when the picture of an acellular nephrosclerosis was seen.

In these rats the changes in the liver were also more
severe. Edema of parenchyma and a marked congestion were
observed. The blood vessel walls were homogeneous, their
lumina were dilated and overfilled with erythrocytes. In
the dilated lumina of the intertrabecular capillaries there
were macrophages containing hemosiderin and polonium. An
albuminoid dystrophy and in certain animals also a fatty
degeneration of the liver were noted. The epithelium of
the biliary passages was swollen and in places had sloughed
off. Polonium was contained in the liver cells in a small
quantity. In the testicles a necrosis of the germinal epith-
elium was noted.

It should be noted that the changes observed of vas-
cular character in the parenchymatous organs of experimental
rats caused us to make investigations of the organs of ani-
mals which had been given (according to the same method)
the single preparation, unithiol. It was shown that in these
animals distinct changes were also observed which were ex-
pressed in a very marked congestion of the organs. Based
on these data it may be supposed that it is completely im-
possible to rule out the effect of unithiol on the vascular
system with this method of application of it.

Conclusions

1. The early use of unithiol according to the calcu-
lation of 100-50 milligram/kilogram from the time of pene-
tration of polonium into the body (0.1 mC/kg) accelerates
and increases the excretion of this element from the body
in a quantitative manner. Polonium is excreted chiefly

through the excretory system (kidneys) where it is concentrated in large quantities. By the same token, the concentration of polonium in the other organs and systems is reduced. This is particularly distinct in all hematopoietic organs (spleen, lymph nodes).

2. The length of life of experimental white rats is increased from 13 to 95 days. The acute course of the disease assumes a chronic nature.

3. Morphological signs of the effect of polonium are delayed in the hematopoietic organs after the use of unithiol and do not have such a severe form (complete disappearance of the formed elements of the blood) as occurred in control rats at the time of their death on the 11th-13th-20th day after giving the subcutaneous injection of polonium (0.1 mC/kg). The macrophagic reaction in experimental rats was reduced, whereby the number of areas producing strong ionization by virtue of the capture of polonium by macrophages was also reduced.

4. In the kidneys, in connection with the accumulation of a large quantity of polonium in them, dystrophic changes develop earlier than in the controls and are of a severe nature (a picture of acellular nephrosclerosis by the 102nd-115th days).

Bibliography

Grodzenskiy, D. "Farmakol. i toksikol.," [Pharmacology and Toxicology], 1946, Vol IX, No 3.
Bacq, Z. M.; Herve, A.; Scherber, F. "Arch. Internat. Pharmacodyn.," 1953, Vol 94, page 102.
Hursch, J. B. "J. Pharmacol. a Exper. Therap.," 1951, Vol 103, page 450.

The Change of Blood Cases in Dogs Affected by Polonium

G. I. Bezin

The aim of the present work is an elucidation of the condition of "the respiratory work of the blood" (a term proposed by G. V. Derviz) in dogs under conditions of acute and subacute radiation sickness produced by polonium. In the literature there are no works devoted to the study of blood gases after involvement of the animal organism by ingested radioactive substances. A number of authors observed a change in the blood gases after X-irradiation (N. N. Blokhin, V. V. Merenov, D. M. Draguzya and A. F. Panin).

The experiments were performed on 12 male dogs weighing 25-30 kilograms. In three dogs there was a cutaneous cannula in the renal vein which had been applied according to the London method in the Pigalev modification. The animals were injected subcutaneously with a neutral solution of polonium (Po^{210}) according to the calculation of 0.05-0.07 mC per kilogram of weight, as the result of which acute radiation sickness developed in six dogs and subacute radiation sickness, in six.

The observation was made of the general condition of the animals, the change in their weight, the concentration of hemoglobin, sedimentation rate, white blood count, red blood count, platelet count and reticulocyte count; the oxygen capacity of the blood, the oxygen content and the carbon dioxide content were investigated dynamically in volumes percent in arterial and venous blood taken from the femoral blood vessels, and in angiostomized dogs, in addition, also from the renal venous blood. In nine dogs the circulation time was determined with lobeline. A complete examination of all these indices was performed before they were given the poison in all animals four or five times. The blood tests were taken on a fasting stomach. Two dogs were kept on a standard diet; the others, on the ordinary vivarium diet. The normal fluctuations in the gas composition of the blood of the first two dogs did not differ from those in the other experimental animals. After the dogs were given polonium the blood for examination was taken one, three, four, seven and nine days, and then at intervals of five or six days. The oxygen saturation of the blood for the purpose of determining the oxygen capacity was accomplished in a Derviz bottle in an atmosphere of air at room temperature. The blood gas concentration was determined on a van Slyke apparatus according to the generally accepted method.

The general clinical picture of radiation sickness in all dogs coincided completely with that described in other
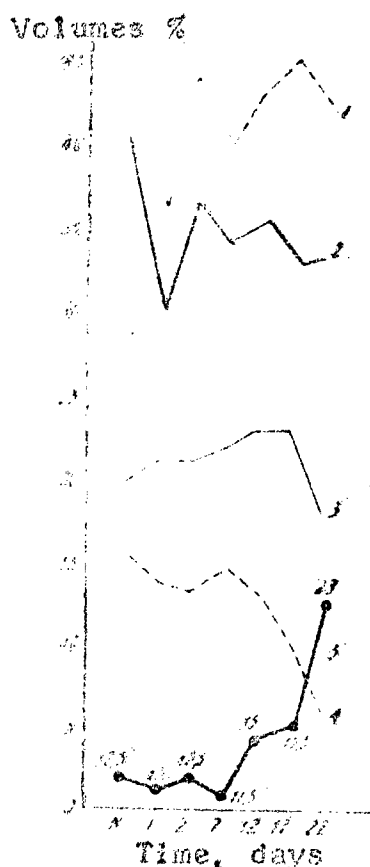
Volumes %

Time, days

Fig. 1. Change in Gas. Content of Blood of Dog Polkan (Acute Radiation Sickness).

1 -- CO_2 concentration in the femoral vein;
2 -- CO_2 concentration in the femoral artery;
3 -- O_2 concentration in the femoral artery;
4 -- O_2 concentration in the femoral vein;
5 -- circulation time (in seconds).

works (A. Lacassagne, I. A. Pigalev and others). In six dogs the acute form of radiation sickness developed with a characteristic marked leukopenia, thrombocytopenia, reduction in the reticulocyte count up to their complete disappearance, and acceleration of the sedimentation rate. A marked reduction in the red blood count and in the percentage of hemoglobin was noted in the dog Gaga. Usually, the red blood count and hemoglobin underwent wave-form variations with a tendency toward a reduction during the last days of life. In the dog Pyatnash an increase in the red blood count and in the percentage of hemoglobin was noted in the terminal stage of the disease. In accordance with these changes the oxygen concentration in the arterial blood of the majority of dogs increased slightly at the beginning of the disease and decreased in the terminal period (Fig. 1). In two dogs (Gaga and Seryy) a marked drop in the oxygen concentration was observed in the arterial blood, which proceeded in parallel with the reduction in the quantity of hemoglobin. The change in the oxygen concentration in the venous blood (femoral vein) was completely different and unitypical for all dogs. As early as the first to third day after injection of polonium a reduction in the oxygen was noted, which continued until the animals died (see Fig. 1).

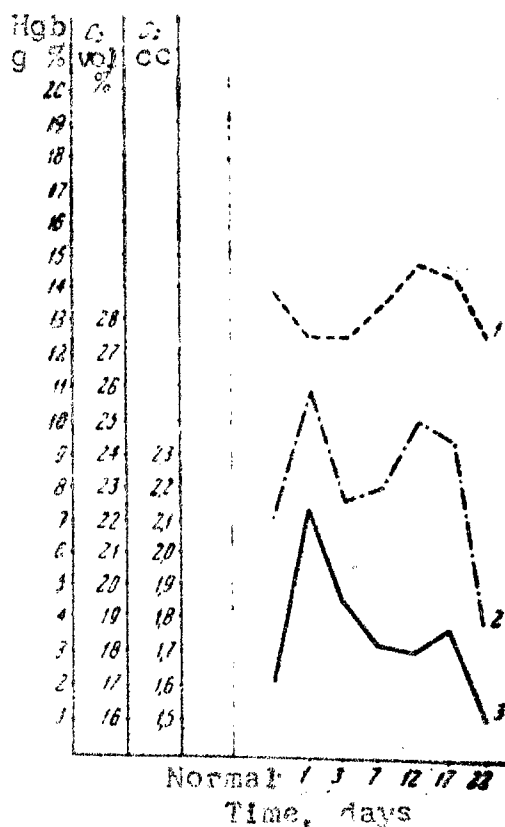The arteriovenous difference with respect to oxygen for the muscles of the hind extremity and the percentage of utilization of O_2 by the tissues increased as early as on the third day after the poisoning and increased with the development of the radiation process. In the terminal period the arteriovenous difference exceeded the

56

Fig. 2. Change in the Hemoglobin Concentration 1, in the Magnitude of the Oxygen Capacity 2, and in the OCI 3 in the Dog Polkan.

original figures by 2 to 2 1/2 times. This index changed in a wave-form manner for the kidneys: at the climax of the disease its magnitude decreased markedly because of an increase in the concentration of O_2 in the renal venous blood, increased in the last few days of life by comparison with the original level, but did not reach the same level as the arteriovenous difference with respect to oxygen for the muscles of the extremity.

In four dogs with an acute course of the process the oxygen capacity of the blood was investigated. In three dogs it increased as early as on the first day after the poisoning, continued to remain at an elevated level, and decreased in the terminal period. In the dog Pyatnash the value of the oxygen capacity varied in a direction of both increase and decrease. The increase in the oxygen capacity noted was associated with the increased capacity of hemoglobin to bind oxygen. In the first three dogs the oxygen capacity index (OCI) and the changed in parallel and in the Pyatnash the OCI was reduced days of the disease, and this

value of the oxygen capacity same direction (Fig. 2). In as early as in the first few decrease became greater until the fatal outcome. However, because the concentration of hemoglobin in this dog increased during the course of the disease the oxygen capacity of the blood remained within limits of normal variations (17.85-20.05 volumes percent) despite the reduction in the OCI. The study of the circulation time in the same four dogs showed that during the first few days the circulation time remained normal or increased slightly. In the second week a lengthening of the circulation time was observed, which progressed, and in the terminal period its magnitude was two or three times less than the original (see Fig. 1).

In six dogs with subacute radiation sickness leuko-
penia, thrombocytopenia, reduction in the reticulocyte
count, increase in the sedimentation rate were observed,
but the intensity of these changes was not so great as in
the first group of dogs. The number of erythrocytes and
the quantity of hemoglobin in the majority of cases was in-
creased or varied within normal limits, and only in the dog
Dik was a decrease in hemoglobin observed beginning with
the end of the first month of sickness.
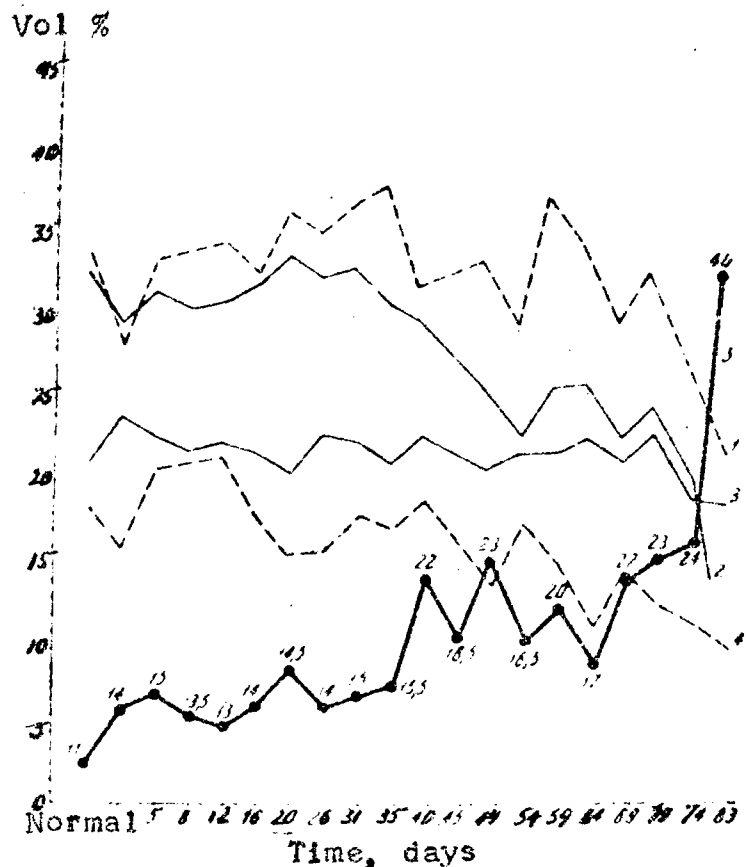
Fig. 3. Change in Gas Composition of Blood in the Dog Dik
         (Subacute Radiation Sickness).
Key, the same as for Fig. 1.

The O_2 concentration in the arterial blood of Trezor
and Geroy was decreased during the first two weeks, and
then a gradual increase in it was observed which was main-
tained also during the terminal period. In the other dogs,
as early as the first to third day after the poisoning the
concentration of O_2 in the arterial blood increased and re-

mained at an increased level throughout the disease (Fig. 3).

The O_2 concentration in the venous blood of the dog Trezor (length of life 45 days) was decreased as early as on the third day. This decrease progressed, reaching very low figures: 10-11 volumes percent at the climax of the disease; two volumes percent on the day of death (normal -- 15.5 volumes percent). In three dogs (Dik, Druzhok, and Geroy) in which the disease lasted longer (length of life 2 1/2 to 3 months) the oxygen concentration in the venous blood underwent wave-form changes, but in the majority of cases it was less than the original figure (see Fig. 3). In two dogs (Kazak, Ryzhiy) changes of opposite character were found: a gradual increase in the O_2 concentration in the venous blood. For example, in Ryzhiy the O_2 concentration on the eighth day was 13.9 volumes percent; on the 33rd day, 14.9 percent; on the 49th day, 22.3 volumes percent; the original value had been 11.5 volumes percent.

The dog Trezor was angiostomized. Throughout the sickness, with the exception of the latent period, the oxygen concentration in the renal blood was higher than the original magnitude. It should be noted that at the climax of the disease (15th-35th day) the arteriovenous difference with respect to oxygen in the kidneys amounted to 0.2-1.3 volumes percent with an original figure of 2.6 volumes percent. In the terminal period (39th-45th day) an increase in the arteriovenous difference was noted, but at this time its magnitude was either similar to the original or exceeded it by only 1 1/2 times.

The CO_2 concentration in the arterial blood during the first three weeks varied chiefly in the direction of a decrease. Then, at the end of the first month of the sickness a steady reduction in the CO_2 occurred in the arterial blood (see Fig. 3). Of all six dogs arterial hypocapnia was not observed only in one (Kazak).

As early as the first to fourth day after the poisoning an increase in the oxygen capacity of the blood was noted in five dogs. During the course of the development of the sickness the magnitude of the oxygen capacity either varied in both directions (Trezor, Dik, Geroy), remaining increased in the majority of cases, or exceeded the original level throughout the sickness (Druzhok, Kazak, Ryzhiy). In the terminal period it dropped to values less than the original (Fig. 4) in only two dogs.

Changes in the OCI proceeded cyclically but were not unitypical. In four dogs (Trezor, Dik, Druzhok, Geroy), an increase in the OCI was noted on the first to third day with a subsequent return to the original figure. In two dogs (Kazak, Ryzhiy) the OCI on the first day was somewhat reduced.

Afterwards, the value of the OCI varied in the wave-form fashion throughout the entire sickness; in four dogs it was increased in the majority of investigations; in two (Geroy, Ryzhiy), reduced (Fig. 4).
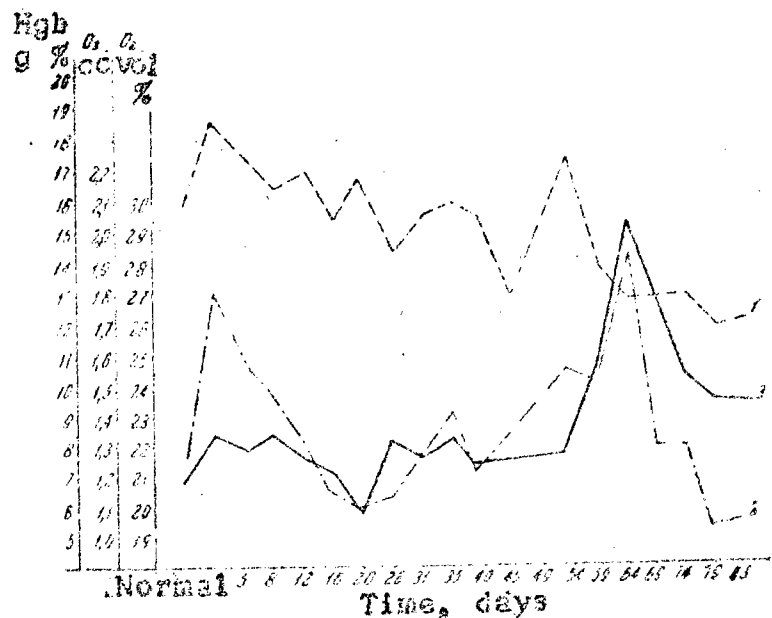
Fig. 4. Change in the Hemoglobin Concentration 1, in the Oxygen Capacity 2, and the OCI 3 in the Dog Dik (Subacute Radiation Sickness).

The circulation time was investigated in five dogs. On the first few days after the poisoning it was essentially unchanged in the majority of animals. By the end of the first month of the sickness a definite lengthening of the circulation time was noted in all animals, and in the terminal period its value was 2 to 3 1/2 times lower than the original figure (Fig. 3).

Both in the acute and in subacute forms of radiation sickness there was no unitypical direction in the changes in arterial blood oxygen saturation. In three dogs with an acute course (the saturation was investigated in four dogs) a reduction was noted in the saturation percentage, and in one dog this occurred throughout the disease, while in two, it was only on individual days during the climax of the sickness. In the subacute form a reduction in the arterial blood oxygen saturation was noted on the third-tenth day in two dogs with a subsequent normalization and a new decrease at the climax of the disease. In three dogs an increase in the percentage of sat-

uration was noted: in one dog no essential changes were found. In the terminal period no marked deviations in saturation were noted.

A reduction in the oxygen concentration in the peripheral venous blood, an increase in the arteriovenous difference with respect to oxygen for the tissues of the hind extremity, an increase in the percentage of utilization of oxygen by the tissues, an increase in the oxygen unsaturation coefficient of capillary blood, as well as lengthening in the circulation time all speak for the fact that during the course of radiation sickness produced by Po^{210} signs of anoxia of the stasis type develop in the body. In cases of the acute course of the process these signs increase more actively and steadily, and in the subacute form they develop in a wave-form fashion, that is, during the course of the disease at various intervals of time a return to the original level may be noted but with an obligatory increase in these disturbances in the terminal period.

As G. V. Derviz, A. A. Tregubov and others have shown, the abnormal prolongation of the respiratory cycles is one of the main signs of development of circulatory anoxia, because in the healthy organism the increased oxygen requirement is satisfied primarily by means of an increase in the minute volume of the heart with an almost unchanged arteriovenous difference. The increase in the arteriovenous difference occurred because of a decrease in the oxygen concentration in the venous blood, which was accompanied by a reduction in the percentage of oxygen saturation of the venous blood. In individual cases the percentage of oxygen saturation of the venous blood in the terminal stage was so low that even under conditions of a maximum change in the dissociation curve of oxyhemoglobin downward and to the right the partial pressure of oxygen in the venous blood must have been lower than the normal figure. The decrease in the partial pressure of oxygen in venous blood below normal is a harmful factor, which indicates that the oxygen diffusion from the capillaries into the surrounding tissues occurs in the presence of an inadequate tension of it in the capillary blood.

As is well known, increased permeability of the blood vessel membranes occupies an important place in the syndrome of radiation sickness. A consequence of this is the outflow of coarsely dispersed colloids -- proteins -- into the tissues, which interferes with the gaseous diffusion and contributes to the development of an anoxic state (B. N. Mogil'nitskiy and M. S. Brumshteyn). In the experiments of S. M. Mikhaylovich in dogs affected by polonium an increasing skin blood vessel permeability was noted with a subacute course

61

of the disease. This made it possible to assume that in our experiments one of the causes of development of circulatory anoxia was the increased vascular permeability.

The arterial hypocapnia observed in our experiments speaks for possible changes in the acid-base equilibrium of the blood. A reduction in the carbon dioxide concentration in the arterial blood may be considered a compensatory reaction for normalization of the acid-base equilibrium. This statement is in agreement with the data of V. L. Nesterova and L. N. Mar'yich, who noted a reduction in the reserve alkalinity of the blood. The increase in oxygen concentration in the venous blood coming from the kidneys noted in angiostomized dogs at the climax of the disease and the marked reduction in the arteriovenous difference with respect to oxygen associated with this may be explained tentatively, on the one hand, by the hemodynamic changes (increase in blood flow, spasm of kidney vessels), and on the other, by disturbances in the tissue respiration in the kidneys (Z. I. Poluboyarinova). There are reports (A. A. Tregubov, G. G. Gazenko and T. I. Abramson, K. M. Malenkova) that the OCI is not a stable index and can be changed both in pathology and in certain physiological stresses.

Increase of the OCI in the majority of our experiments may be regarded as a manifestation of a compensatory reaction which provides for a better oxygen supply of the tissues in the presence of anoxia. The variability of the OCI depends on substances contained in the erythrocyte itself, on the condition of its stroma, the presence of decomposition products of protein bodies in the body, which is reflected in the oxygen-combining properties of hemoglobin. From numerous works it is well known that the radiation syndrome is accompanied by the accumulation of protein decomposition products in the body. It is also well known that the erythrocyte membrane itself undergoes essential changes (A. S. Mochalina). On the basis of this it seems possible to associate the increase in the OCI value with these changes in our experiments.

Conclusions

1. In the acute form of radiation sickness produced by Po^{210} signs of circulatory anoxia arenoted beginning with the second week, and in part of the cases, anoxemic anoxia.
2. In the subacute form, in the third to fourth week signs of circulatory anoxia appear, and in individual cases, histotoxic and anoxemic anoxia.
3. In the acute form, beginning with the first week and in the subacute form beginning with the end of the first

month, arterial hypocapnia develops.

4. In the acute and subacute forms of radiation sickness an increase is noted in the magnitude of the oxygen capacity of the blood, as a rule, beginning with the first week, which is associated with a change in the functional properties of hemoglobin.

5. Differences in the change in the oxygen arteriovenous difference for the kidneys and for the tissues of the hind extremity may indicate a dissimilar course of the hemodynamic and metabolic changes in the kidneys and extremity tissues.

Bibliography

Blokhin, N. N. "Vestn. rentgenol. i radiol." [Herald of Roentgenology and Radiology], 1938, Vol 20, page 103.

Gazenko, G. G.; Abramson, T. I. "Byull. eksper. biol. i med." [Bulletin of Experimental Biology and Medicine], 1941, Vol 12, Nos 5-6, page 275.

Derviz, G. V. and others. "Klin. med." [Clinical Medicine], 1947, No 4, page 22.

Draguzya, D. M.; Panin, A. E. In the book: "Material of the Scientific Conference on the Problem of 'Radiation Sickness'". Leningrad, 1957, page 329.

Malenkova, K. M. In the book: "Problems of Roentgenology". Moscow, 1952, page 298.

Mar'yich, L. N. "Proceedings of the Scientific Conference on the Problem 'Early Mechanisms of Radiation Injury'". Khar'kov, 1958, page 23.

Merenov, V. V. Works of the All-Union Conference on Medical Radiology. "Eksper. med. radiol." [Experimental Medical Radiology], Moscow, 1957, page 146.

Mikhaylovich, S. M. Proceedings of the Conference on Medical Radiology. Moscow, 1957, page 10.

Mogil'nitskiy, V. N.; Brumshteyn, M. S.; "Arkh. pat." [Archives of Pathology], 1946, Vol 8, No 3.

Mochalina, A. S. Works of the All-Union Conference on Medical Radiology. "Eksper. med. radiol." Moscow, 1957, page 68.

Nesterova, V. I. In the book: "Pathological Physiology of Acute Radiation Sickness." Moscow, 1958, page 152.

Pigalev, I. A. In the book: "Problems of Pathology and Metabolism." Leningrad, 1950, page 13.

Pigalev, I. A. In the book: "Biological Effect of Radiation and the Clinical Aspects of Radiation Sickness." Moscow, 1954, page 76.

Poluboyarinova, Z. I. Works of the All-Union Conference on Medical Radiology. "Eksper. med. radiol." Moscow,

1957, page 190.

Tregubov, A. A. Disturbance of the Respiratory Function of the Blood in Certain Pathological Processes. Leningrad, 1957.

Lacassagne A. and others. "J. radiol. et electrol.," 1925, Vol 11, page 67.

The Reactivity of the Blood Vessels Under Conditions Where the Organism Is Affected by Polonium

V. V. Vasil'yevskaya

A large part in the pathogenesis of radiation sickness belongs to the vascular disturbances (hemorrhagic syndrome, hypotension, disturbance in the blood supply of the tissues). Under the influence of ionizing radiation the blood vessels lose their capacity of maintaining the normal tone of the wall, to some degree, and that of responding with appropriate changes in the lumina to stimulation. Evidence of this which has been noted in a number of works is the change in the reactivity of the skin capillaries (N. A. Skul'skiy, Muck) and of the ear vessels (V. V. Zakusov, N. V. Lazarev and A. P. Lazareva, Gerstner and others, Gehrels and others) after the effect of ionizing radiation. However, these data were obtained after local irradiation of an area being studied or even after irradiation of tissue separated from the body.

The change in the reactivity of the blood vessels under conditions of the effect of ionizing radiation on the whole body was shown recently in works of R. M. Lyubimova, L. D. Klimovskaya, V. V. Yakovleva, N. P. Smirnova. Only in the work of R. M. Lyubimova was a radioactive substance used, radiothorium, which was injected subcutaneously in doses which produce an acute injury of the body. In experiments on the isolated ear of a rabbit, R. M. Lyubimova noted a markedly increased reactivity of the peripheral blood vessels with respect to adrenalin during the period of pronounced hypotension.

We decided to investigate the reactivity of the peripheral vessels chiefly with respect to the vasodilating effects under conditions where the body is affected by a radioactive substance -- polonium. Polonium was injected subcutaneously (activity -- 0.07 μC per gram of weight), which caused an acute injury to the body which terminated in the death of the animal after an average of three weeks. Observations were made on the ears of 139 rats. Local effects of heat and cold and massage were used, as was also a total body heating and the intraperitoneal injection of acetylcholine. The reaction of the entire vascular network, chiefly of the capillaries, was evaluated visually, and the reaction of the medium-sized blood vessels (central vessels of the ear), thermometrically. For this purpose the junctions of two thermocouples (copper-constantan) were put onto the outer surfaces of the right and left ear respectively, exactly over the central vein. The fluctuations in tempera-

ture characterized the changes in the vascular lumen and of the blood flow through it.

The heat stimulus which was designed on the type of a blowtorch was applied with a constant temperature of 40 or 50° to the outer portion of the left aural concha at the site of branching of the small vessels; the stimulation lasted one minute (checking showed that when the experiment was performed in this way no notable effect of heat radiation on the thermocouple junction was noted). When the temperature of 40° was used the skin temperature over the blood vessels was increased by two to three degrees in the first minute, after which a quite rapid decrease in temperature occurred, and the original level was reached four to five minutes after the effect. With the effect of 50° the temperature over the blood vessels was increased by three to four degrees, and the decrease in temperature occurred more gradually, and the original level was achieved in 10-15 minutes. Beginning with the seventh to tenth day after the injection of polonium the initial temperature over the blood vessels in many cases was lower than normal; the blood vessels of the ear in the majority of cases looked narrow, and the general background was pale; the small blood vessels were in a spastic condition.

The reaction of the blood vessel to the local effect of temperature of 40° in animals injured by polonium was not much changed in its strength; however, in many cases the temperature after recovery to the original level continued to drop and by the 10th-15th minute decreased by one degree, that is, the blood vessels constricted. Less often, the temperature did not drop to the original level for a long time (Fig. 1). After the effect of a temperature of 50° the reaction of the blood vessels in the animals affected by polonium was also well expressed; however, in many rats the return of the temperatures to the original level was delayed (7th and 15th day). In a number of cases on the 10th day the reaction was shortened and somewhat reduced. One rat was examined on the 15th day two hours before death. The blood vessels of its ear, although they were in a state of spasm (skin temperature over the blood vessels was 22° instead of $26-28^\circ$ as in the normal) produced a short but quite pronounced reaction, after which the temperature dropped by one degree compared with the original, and the blood vessels closed down completely.

Therefore, the large vessels of the ear of rats after being affected by polonium do not lose their capacity of responding with dilatation in response to a local heat effect; however, the second phase of the reaction is altered in the majority of cases: the blood vessels either remain

66

dilated for a long time or, after their original condition, continue to constrict.
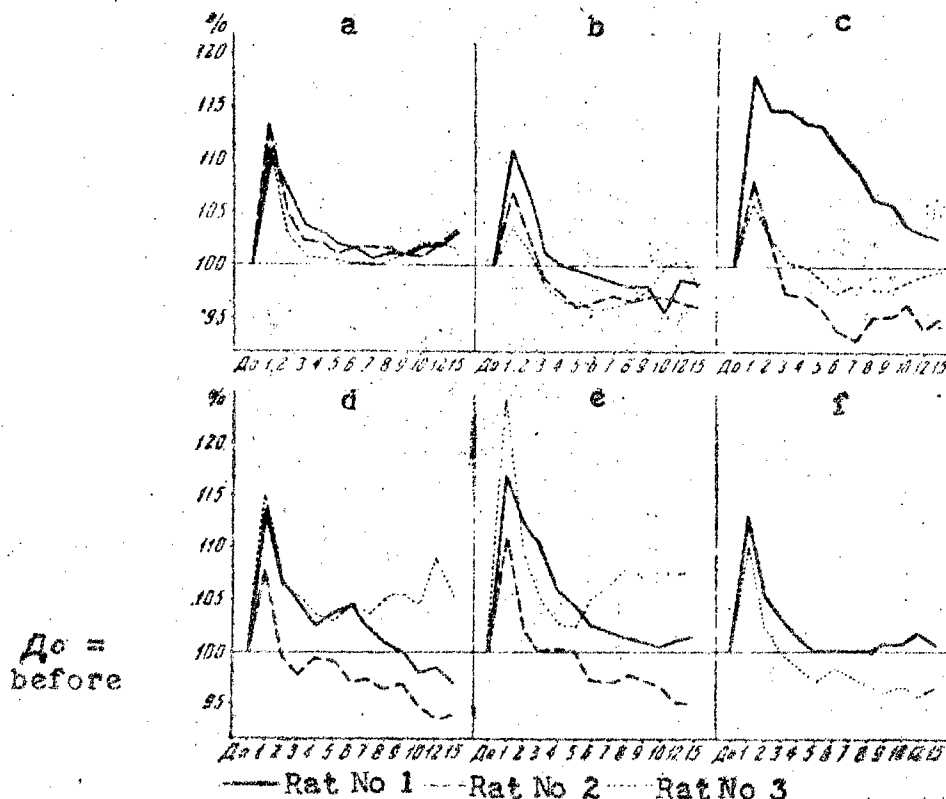
Fig. 1. Reaction of Ear Vessels of Rat to Local Effect of Heat of 40° Before and After Injury With Polonium.

a -- control; b -- the first day after the effect; c -- on the third day; d -- on the seventh day; e -- on the tenth day; f -- on the fifteenth day. Along the abscissa axis the time of observation is plotted in minutes; along the ordinate axis, the temperature changes over the blood vessel expressed in percentages of the original temperature.

In the second series of experiments, an effect on the entire ear was used; the reaction of the blood vessels, chiefly of the small blood vessels and capillaries, was evaluated visually. The ear was put in water for two minutes which had been heated to 43 or 50° or cooled to 8°. In addition to the temperature influences, a mild massage of the ear concha with a cotton tampon was used and made, as much as possible, standard.

In control animals after heating the ear in water the dilatation of all blood vessels begins immediately and

67

the background becomes pink. The reaction, gradually becoming weaker, is maintained for 15-20 minutes.

After putting the ear in cooled water a dilatation of the central vessels occurs first, but after one minute a constriction of all blood vessels begins; the reaction lasts 20-30 minutes. After massaging the ear a mild dilatation of the blood vessels occurs; after four or five minutes the blood vessels return to normal.

On the first and second day after the injection of polonium the blood vessels of the aural concha of the rats produce a considerable and prolonged reaction both to heat and to cold. When the ear is put in water with a temperature of 50° the background becomes dark pink, the blood vessels dilate markedly. After the effect of cooled water the constriction is expressed markedly also. The reaction often becomes inverted in response to weak stimuli (43°, massage), that is, the blood vessels react with constriction rather than dilatation. In certain cases the same kind of reflex reaction occurs in the opposite (intact) ear.

Beginning with the third, but usually beginning with the seventh day after the injection of polonium the reaction of the blood vessels of the ear becomes weaker, particularly in response to heat stimuli. In a number of cases the blood vessels react to cold with a marked constriction, but sometimes the reaction is weakened. The reaction is often absent or inverted in response to a temperature of 43° and massage. In the opposite ear a reflex reaction is observed in the form of a constriction of the blood vessels. Beginning with the 10th-15th day and later, the reaction of the blood vessels to stimulation, as a rule, is sluggish and prolonged; recovery does not occur during the observation time (30 minutes). Gradually the tendency of the blood vessels to react with constriction to various stimuli, including vasodilatory stimuli, increases. Only in response to a temperature of 50° can a sluggish dilatation of the blood vessels be observed and then sometimes after a temporary constriction. Almost in all cases of stimulation a reflex constriction of the blood vessels of the opposite (intact) ear is observed.

In the third series of experiments a total body heating was used with a reflector focused on the trunk and paws of the rat (with a protection of the head) for five minutes; thereby, a temperature of 27° was created at the level of the surface of the trunk. With the use of this total body effect on the organism the skin temperature over the central vessels of the ear increased by $2.5-3^\circ$ (maximum in the fourth-fifth minute) but immediately after turning off the lamp it decreased. In rats affected by polonium

the reaction of the ear vessels was absent in a number of
cases; sometimes a certain delay in the reaction could be
observed, with lengthening of it and even a paradoxical
reaction (Fig. 2).

Fig. 2.   Reaction of Ear Vessels of Rat to Total Body
Heating of Trunk Before and After Injury With
Polonium.

a -- control; b -- on the first day after the effect;
c -- on the third day; d -- on the seventh day; e -- on
the tenth day; f -- on the sixteenth day.

In the fourth series, we used the intraperitoneal
injection of 0.8 cubic centimeter of one-percent acetyl-
choline solution. In the animals an external reaction was
observed in response to the acetylcholine; lacrimation and
salivation. The reaction of the ear vessels in healthy
rats was expressed in a dilatation of the blood vessels --
the skin temperature rose by one to two degrees during the
first two minutes, after which a gradual decrease in it to
the original level occurred. The reaction was most often
biphasic; the second wave was somewhat weaker and came

about in the 8th-14th minute. During the period between
the waves the temperature dropped to normal or remained at
a comparatively high level.

In the rats injured by polonium the external reaction
to the injection of acetylcholine was well expressed. The
vascular reaction in response to the injection of acetyl-
choline in the rats injured by polonium, beginning with the
first-third day, but particularly distinctly beginning with
the 10th day, decreases, in certain cases is inverted, the
second wave of dilatation is not expressed as a rule, and
often a considerable constriction of the blood vessels
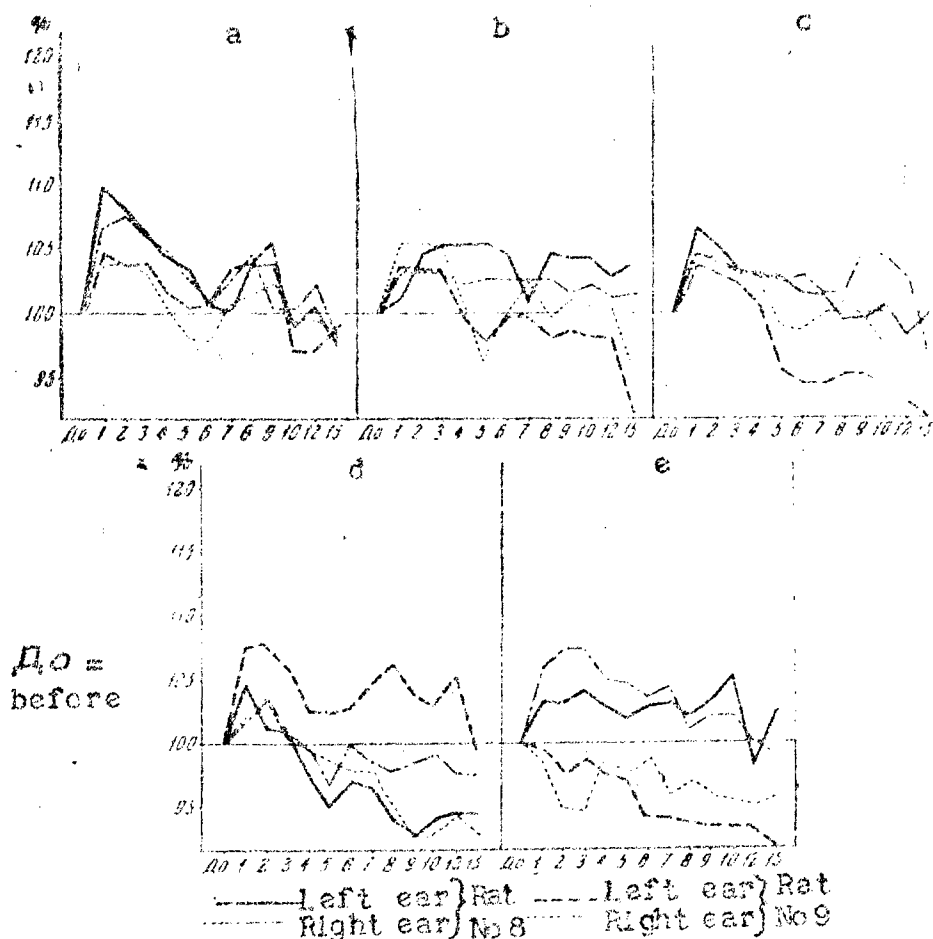occurs in place of it (Fig. 3).

Fig. 3. Reaction of Ear Vessels of Rat to Intraperitoneal
Injection of 0.8 Cubic Centimeter of One-Percent
Solution of Acetylcholine Before and After
Polonium Injury.

a -- control; b -- on the third day after the effect; c --
on the seventh day; d -- on the tenth day; e -- on the
fifteenth day.

70

It should be noted that after the total-body effect --
injection of acetylcholine, heating of the body of rats --
the right and left ears often respond differently; sometimes
one ear gives a considerably weaker reaction than the other,
that is, an asymmetry is observed.

This phenomenon was noted both in control rats and in
intoxicated rats, whereby in the latter the reaction was
opposite in several cases: the blood vessels of one ear di-
lated; those of the other constricted.

Observation of the blood vessels of the ear of animals
injured by polonium after the effect of a number of stimuli
showed that the reaction of the vessels to vasodilating
agents is decreased, sometimes down to the point of disap-
pearance. At the climax of the disease, the blood vessels
often go into a state of constriction under the influence of
various vasodilating stimuli -- either a negative phase of
the reaction is observed or a paradoxical reaction. The
changes deal primarily with the capillaries the disturbances
of which are pronounced beginning with the first few days.
The predominance of the vasoconstrictor tone leads to the
fact that the blood vessels are chiefly in a constricted
condition. As a result of this the blood supply of the
tissues is impoverished, their trophism is impaired, and the
capacity of withstanding harmful influences is reduced.

Changes in the relationships of the vasoconstrictors
and vasodilators in radiation sickness are determined, evi-
dently, primarily by the neuro-humoral disturbances in the
body. However, at the present time it is difficult to
imagine the specific mechanism of the changes obtained. At
autopsy of the animals injured by polonium during a period
when constriction of the peripheral blood vessels was noted
we found a dilatation of the capillaries of paralytic type
in the kidneys, liver and lungs, and the number of capillaries
was increased. Evidently, the reactivity of the blood vessels
of internal organs under conditions of radiation sickness is
changed in a different way from the reactivity of the skin
vessels. Possibly the constriction of the peripheral vessels
is to some degree a compensatory reaction to the decrease in
the blood vessel tone in the abdominal cavity and the occur-
rence of hypotension noted after radiation injury.

Conclusions

1. The reactivity of the blood vessels of the ear of
rats under conditions of acute injury by polonium is altered,
whereby the changes in capillary reactivity are most distinct.

2. The weakening and inversion of the blood vessel
reaction in the ear in response to vasodilating effects are

71

noted beginning with the first day after the injection of
polonium, but are particularly distinct during the period of
the greatest development of the process of injury, that is,
beginning with the tenth day.

Bibliography

Zakusov, V. V. "Vrach. delo" [Physician's Affairs], 1924,
    No 20, 23, page 1087.

Lazarev, N. V. and Lazareva, A. P. "Strahlentherapie," 1926,
    No 23, page 41; Vol 25, page 255, 458; "Vrach. delo,"
    1928, No 1, page 25.

Skul'skiy, N. A. "Vrach. delo," 1926, Nos 10-11, page 903.

Yakovlev, V. V. Proceedings of the All-Union Scientific
    Technical Conference on the Use of Radioactive and
    Stable Isotopes and Radiation in the National Economy
    and in Science. Moscow, 1957, page 20.

Gehrles, P. E.; Heite, H. J. and Otzen, P. "Strahlentherapie,"
    1956, Bd. 100, S. 129.

Gerstner, H. B.; Brooks, Ph. M. and Smith, S. A. "Am. J.
    Physiol.," 1955, Vol 182, page 459.

Muck, O. "Strahlentherapie," 1939, Bd. 65, S. 449.

Siedamgrotzky, K. Ibid, 1925, Bd. 19, S. 84.

Morphological Changes in the Peripheral White Blood Element After a Total-Body X-Irradiation in Combination With a Wound of the Kidney

O. L. Tiktinskiy

We made investigations on irradiated animals with gunshot wounds of the kidney. Dogs and rabbits were used in the experiment. During the first hour after a total-body single irradiation with X-rays of dogs in a dose of 350 r and of rabbits in a dose of 500 r, the gunshot wound was inflicted on the kidney. All the wounded animals were given an organ-saving operation -- resection or suture of the kidney. The blood was examined no less than twice before the experiment, on the second and fifth day after the experiment, and subsequently once a week for two months in animals with a mild course of the disease and every two or three days in animals with a severe course.

The experiments were performed on 51 dogs and 41 rabbits. Part of the wounded animals was not irradiated, One group of irradiated animals was not wounded. Penicillin treatment was given to half of the animals during the 10 days after the experiment.

Changes in the peripheral white blood morphology amounted chiefly to the following. On the second day after the experiment, a high degree of leukocytosis was noted in the majority of irradiated wounded animals because of an increase in the number of stabs and segmented neutrophils. In the other animals the white blood count per cubic millimeter of blood either increased very slightly or was reduced on the second day. In those animals in which there was no primary leukocytosis during the first one or two days a clinically expressed hemorrhagic syndrome developed subsequently, and they all died from radiation sickness. By the fifth day after the experiment, a leukopenia occurred in the majority of animals, depending on the severity of the sickness, down to limits of 4400-300 white blood cells per cubic millimeter of blood. In the animals which survived (not treated by penicillin), the normal leukocyte count in the blood was restored: in dogs, after two-six weeks; in rabbits, after two-eight weeks; in the surviving animals (treated with penicillin) -- one and a half-six weeks. In the blood of animals which died from acute radiation sickness aggravated by the wound leukopenia was maintained until the day of death. In certain animals, particularly in rabbits, a leukocytosis was noted in the second week after the experiment, which was again replaced by leukopenia. All these animals survived.

73

In the series of control irradiated animals a slight increase in the leukocyte count was noted in two dogs and two rabbits on the second day after irradiation (by five to ten percent). On subsequent days the white blood count dropped to 5400-1000 per cubic millimeter of blood and returned to normal in the surviving animals after one to four weeks. In the animals which died, leukopenia existed until the day of death.

In the wounded non-irradiated animals there was a high degree of leukocytosis on the first to second day after the wound was inflicted: in dogs -- 15,000-28,000; in rabbits -- 13,000-17,000 white blood cells per cubic milli-meter of blood. Beginning with the third to fourth day, a reduction in the white blood count was noted. After two to six weeks in dogs and two to eight weeks in rabbits, it became normal. The lymphocyte count (most sensitive to the effect of ionizing radiation) decreased markedly on the first to second day after irradiation in the wounded irradiated animals. By the fifth day almost a complete recovery of the normal lymphocyte count was noted in more than half of the surviving dogs. The absolute lymphocyte count, particularly in the animals which died, frequently fluctuated within broad limits: from 10 cells to 1000 per cubic millimeter of blood. The relative monocyte count during the first two days after the experiments decreased; after two or three weeks it increased above the original in the surviving dogs, and by the end of the second month it came to normal.

The differential count changed in the rabbits almost in the same way as in the dogs. The initial leukocytosis developed chiefly because of an increase in the number of all the granulocytes. Among the neutrophils there were fewer young forms than in the dogs. The lymphocyte count varied to a lesser degree. After six to eight weeks there was a persistent lymphocytosis in almost all the rabbits. In two rabbits which died from radiation sickness, fragmen-tation and cytolysis of the leukocytes were noted a day before death.

In the irradiated wounded animals treated with peni-cillin an increase could be noted in the relative eosinophil count by two to five times in two-thirds of the surviving dogs three to four weeks after irradiation. A relative increase in the eosinophil count by one and a half to three times occurred in six out of nine surviving rabbits of the third series. In one rabbit, the eosinophil count decreased. An absolute and relative lymphocytopenia during the first one or two days was noted also in the control irradiated animals, and then the lymphocyte count increased, and by the fifth day, as a rule, it returned to normal. In seven dogs

74

and three rabbits (wounded, non-irradiated) there was a slight relative lymphocytopenia after the wounding which promptly disappeared, and after three to four weeks it was replaced by relative lymphocytosis. In the group of those treated with penicillin, there was a moderate eosinophilia.

In the majority of cases in dogs with combined afflictions, the sedimentation rate was determined on the fifth day after the experiment, depending on the severity of the wound; this was accelerated to varying degrees (up to 80 millimeters in an hour). Not uncommonly, marked fluctuations were noted in the sedimentation rate (by several scores of units) during a single week, which could not always be tied in with the clinical picture. In the control irradiated animals, the sedimentation rate was accelerated to a lesser degree (up to 26 millimeters in an hour). In the wounded non-irradiated animals, the sedimentation rate reached 47 millimeters in an hour, and, for the most part, corresponded to the severity of the wound.

Therefore, the morphological picture of the peripheral white blood element after a total-body X-irradiation combined with a kidney wound in experiments on animals was, for the most part, the same as in radiation sickness without kidney injury. The more pronounced neutrophilic leukocytosis during the first few days after the injury in the surviving animals, the persistent leukopenia until the day of death, and the absence of a phase of primary leukocytosis in animals with fatal outcomes, eosinophilia in those treated with penicillin, and in a number of cases leukocytosis during the period of the climax of radiation sickness, which is a good prognostic factor, should be noted.

Bibliography

Yegorov, A. P. In the book: "Biological Effect of Radiation and the Clinical Aspects of Radiation Sickness." Moscow, 1954, page 15.

Yegorov, A. P. and Bochkarev, V. V. Hematopoiesis and Ionizing Radiation. Moscow, 1955.

Kurshakov, N. A. "Klin. med." [Clinical Medicine], 1955, No 6, page 12.

Mokrovskaya, S. P. "Med. radiol." [Medical Radiology], 1957, Vol 2, No 1, page 64.

Rubinshteyn, Ya. G.; Nemkin, M. N. and Shishlyannikova, L. I. "Voyen.-med. zhurn." [Military Medical Journal], 1957, No 6, page 33.

Sokolov, S. S. "Vestn. rentgenol. i radiol." [Herald of Roentgenology and Radiology], 1956, No 1, page 30.

Filatov, V. I. "Voyen.-med. zhurn.," 1957, No 6, page 37.

CRITIQUE

H. O. Davidson. Biologicheskiye deystviya obshchego γ-oblu-cheniya na lyudey. [Biological Effect of Whole-Body Gamma-Radiation on Human Beings]. 1957.

In this monograph the basis and examples are given for the practical use of Blair's theory with various modifications concerning the influence of γ-radiation on people under conditions of application of atomic weapons. The work is of great practical interest for military men, workers in the MPVO [Local Antiaircraft Defense] service as well as for radiobiologists and persons in contiguous specialties. The basic principle of Blair's theory is as follows: radiation injury develops in proportion to the dose rate, and recovery occurs spontaneously at a rate proportional to its magnitude, while the degree of residual irreversible injury is proportional to the total whole-body dose.

Both under conditions of military operations with the use of atomic weapons and for measures protecting the population against the effect of γ-radiation in a focus of an atomic explosion it is extremely essential to be able to determine rapidly the magnitude of the radiation dose which a person may receive without risk of a fatal outcome or the magnitude of the dose which can produce only a mild degree of radiation sickness or affliction without external manifestations. However, the magnitude of these doses will be different depending on the intensity of radiation and the duration of the intervals of time between the various radiations in the case of fractional radiation. For example, a dose of 600 r when given as radiation over a short period of time is fatal for man, but if this dose is received by a person fractionally, reaching the total after three irradiations with intervals of several days between the individual radiations, the fatal outcome is excluded. This fact has been made the basis of the so-called conception of "pure injury." The essence of it consists of the fact that the degree of radiation affliction of the body will gradually decrease by virtue of recovery processes; therefore, if irradiation is fractional or considerably protracted in time the magnitude of the injury will be determined by the magnitude of the total dose, on the one hand, and the magnitude and strength of the recovery processes of the body, on the other. Hence, the degree of "pure injury" will be greater after a person has received a dose of 600 r in a single, brief radiation than in the event this dose is received through multiple administrations of ionizing radiation. On the basis of mathematical analysis of the experimental

radiobiological material, the author has shown that the recovery process is of an exponential nature, and that it will be different for different species of animals in one day, whereas the percentage of "irreversible injury" for all species of mammals (mouse, rat, guinea pig, donkey, dog, man) amounts to 10, on the average.

The following example is presented. If three groups of white mice are irradiated with a semilethal dose of 518 r [the LD_{50} dose] but given in two administrations of 260 r each with intervals of 2, 10, and 20 days between the radiations, then in order to obtain the death of 50 percent of the mice (that is, the effect equivalent to a dose of 518 r) the repeated dose has to be increased respectively to 305, 435 and 476 r. This may be regarded as a gradual reduction in the degree of injury from the primary radiation given in a dose of 260 r; after two days the degree of injury is equivalent to a dose 215 r (305-260 = 45); after 10 days, to a dose 83 r (435-260 = 175), and after 20 days, only to a dose of 42 r (476-260 = 216).

Here, the author does not place any equal sign between the nature and the degree of injury from a dose of 42 r and the dose of 260 r 20 days after the effect. In other words, he considers them as not equivalent biologically and in a special chapter, "The Late Effects of Irradiation," he even concludes that while the magnitude of the early effect (different degrees of radiation sickness and mortality rate) is determined by the effective dose, the late effects, for example, the frequency of occurrence of leukemias, are determined by the magnitude of the total dose (frequency of occurrence of leukemias in inhabitants of Hiroshima and Nagasaki are directly related to their location with respect to the epicenter of the explosion). The time from the moment of a single radiation to the moment when the degree of "pure injury" is equivalent to half the dose of the original radiation is designated by the author as the "recovery half-life." It has been shown that the recovery half-life can be correlated with the length of life of its leukocytes. Thus, the recovery half-life of white mice amounts to 3-8 days; of rats, 6-9 days; of dogs, 14-18 days; of the donkey, 20-28 days; of man, 25-35 days. The degree of recovery in man amounts to 0.001 in one hour (β factor), and the magnitude (γ factor) of irreversible injury, to 0.1.

Later, the author shows in detail how these radiobiological data can be utilized in practice in the matters of planning and arrangement of protective adaptations (shelters) and in the matter of carrying out military operations. In the author's opinion, the principal directions in which this material may be used are the following:

77

1) the utilization of shelters in contaminated areas with the aim of sheltered during the period with an initial high radiation intensity; 2) evacuation from areas which are under threat of radioactive fallout; 3) the basis of the possibility of brief radiation in carrying out emergency work in checking afflicted persons and in rescuing them; 4) renewal of the usual activity in contaminated areas during the period of residual radioactivity.

Before analyzing these problems the author gives detailed definitions of the various units of measurements and arbitrary designations.

1) R_1 -- the intensity of radiation an hour after the blast at a point three feet from the surface of level ground; it is measured in roentgens per hour. The radiation intensity after time t_2 after the blast (R_2) is determined from the equation:

$$R_2 = R_1 t_2^{-1.33} ;$$

2) P -- the protection factor, which represents the ratio of the radiation intensity in an open field to the intensity in a given type of shelter; obviously, in the open field $P = 1$;

3) D_e -- the effective dose, that is, the dose which produces the same magnitude of effect of early injuries or the same degree of "pure injury" as a single momentary irradiation with a certain dose;

4) D_{e1} -- the unit of effective dose; the effective dose which may be obtained when $R_1 = 1$ r/hour and $P = 1$; then $D_e = (R_1/P) \cdot (D_{e1})$;

5) D_c -- the cumulative dose; the total number of roentgens received from continuous prolonged radiation or from multiple brief exposures; D_c is never less than D_e; however, during the course of the first two days after the blast or after the same chronic exposure D_c and D_e are essentially equal to each other;

6) D_p -- the constant dose; a fraction of part of the cumulative dose which brings about the "irreversible injury."

$$D_p = 0.1 \cdot D_c ;$$

7) t_1 -- the time of entry of a person into a contaminated area or the time of fallout of radioactive sediment on a locality in which the given person is located; it is measured in hours after the time of the blast;

8) t_2 -- the time of leaving a locality contaminated by radioactive fallout; it is measured in hours after the time of the blast;

78

9) $t_2 - t_1$ -- the duration of irradiation.

All these arbitrary designations and units of measurement are necessary for finding the magnitude of the effective dose (D_e), because only it determines the degree of the early radiation effects.

A graphic analysis of the change in the values of D_c, D_e and D_p presented in the monograph shows that with a continuous irradiation and with $t_1 = $ one hour, the value of D_c during the first four days after the blast is very close to the value of D_e, so that the cumulative dose can be utilized for evaluating the effect of irradiation. After a longer period (more than four days) D_e is considerably less than D_c; therefore, for the purpose of evaluating the irradiation effect use must be made of the effective dose. The effective dose needs to be determined also after repeated irradiations.

Therefore, the effective dose comes close to the cumulative dose when there are great radiation intensities or when the intervals between the individual irradiations are very short, and, conversely, it becomes much less than the cumulative dose when there are small radiation intensities or when the intervals between the individual irradiations are quite prolonged.

Since the radiation intensity of radioactive fallout is steadily decreasing, according to the equation $R_2 = R_1 \cdot t^{-1.33}$, and the degree of protection of people in the area of radioactive fallout may be different, the value of the effective dose will be determined not only by the intensity of radiation at a given point and the duration of radiation $(t_2 - t_1)$ but also by the protection (P). In the book, a number of graphs is presented (Figs. 1, 2) which make it possible rapidly and easily to find the degree of protection for a given effective dose at a given radiation level and a known $t_2 - t_1$ or, on the other hand, the magnitude of the effective dose with a given degree of protection (type of shelter), with a given level of radiation and a given $t_2 - t_1$ (examples 1 and 2). In addition, the duration of stay in a contaminated area (that is, $t_2 - t_1$) may be computed according to the given effective dose with a given type of shelter and radiation level (example 3). By means of these graphs it is also easy to determine the percentage of recovery different numbers of days from the end of the preceding irradiation (Fig. 3) or the time after the blast at which normal activity may be resumed in a contaminated area without the risk of exceeding the given effective dose, for example, 50 r (example 4).
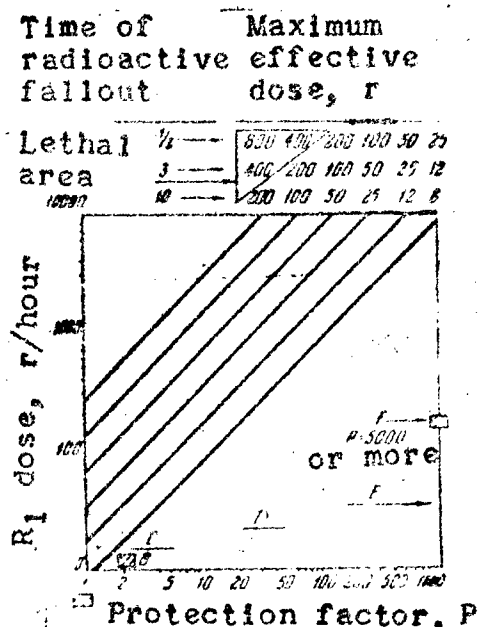
Fig. 1. Value of Protection Factor Needed for Limiting the Maximum Effective Dose to the Established Limit.

A -- walking or motionless state in open field; B -- on horse or in a passenger automobile; C -- in housing or corresponding structures without a substructure; D -- substructure of house (without any essential damage); E -- inside a concrete building of many stories (there may be slight external damage); F -- special shelter covered with a minimum three-feet thick layer of earth or with an equivalent covering.

Example 1. Radioactive fallout at three arbitrary places occurred 0.5, 3 and 10 hours after the blast, and the radiation intensity (R_1) in this time amounted, respectively, to 1000, 300 and 50 r/hour. The dose of 200 r is taken as the maximum effective dose which permits 100 percent survival. What should the value of the protection factor be in each of the three arbitrary points in order to provide 100 percent survival?

Solution. In the upper part of Fig. 1 we find the maximum effective dose -- 200 r -- opposite the times indicated, and then we go down along the corresponding diagonal line to the dose level 1000, 300 and 50 r/hours; from these points we draw a vertical line downward to the places where they intersect the abscissa axis, where we find the values of the protection factor (type of shelter). For these points, P would be equal, respectively, to 15, 2 and 1.

Example 2. Let us assume that the personnel has a shelter of type D. The radioactive fallout reached this

80

shelter after a half hour. What intensity of radiation is
needed to produce fatal outcomes?

Solution. The magnitude of the protection factor
for a type D shelter is within limits of 20-50. Let us
take the lower value of P (20), and from this point let us
construct a perpendicular to the point of intersection with
a diagonal line corresponding to the time, 1/2 hour, and
the effective dose of 200 r. From this point let us draw a
horizontal line to the point of intersection with the ordi-
nate axis. At the point of intersection we find the dose
1400 r. Therefore, with an intensity of 1400 r/hour, fatal
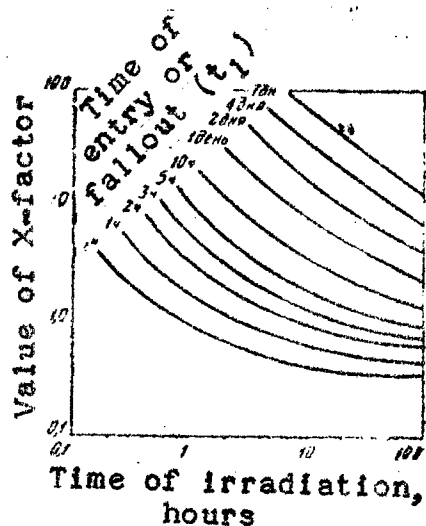outcomes can occur in this shelter.

y = hours

$\partial H я$ = days

Fig. 2. Maximum Permissible Irradiation Time $X = R_1/D_e P$.
D_e -- established level of effective dose in roentgens; R_1
-- radiation intensity at time t_1; P -- value of protection
factor.

Example 3. Emergency work needs to be carried out
in a contaminated area where R_1 = 2000 r/hour, P = 4. The
effective dose (D_e) should not exceed 150 r. If the time
of entry into the contaminated area (t_1) amounts to 10
hours, what should the maximum permissible time spent in
the area be?

Solution. First of all, let us find the values of
the X-factor for these conditions: $X = R_1/D_e \cdot P = 2000/150$
x 4 = 3.3.

Afterwards, using the graph presented in Fig. 2, let
us make the following construction. Let us draw a horizon-
tal line through the point where X = 3.3, and from the
point of intersection of this horizontal with the line which

corresponds to 10 hours (t_1), let us drop a perpendicular to the abscissa axis. At the site of intersection of this perpendicular with the abscissa axis we find the figure 10. Therefore, under these conditions one can stay in the contaminated area for 10 hours, whereby D_e will not exceed 150 r.
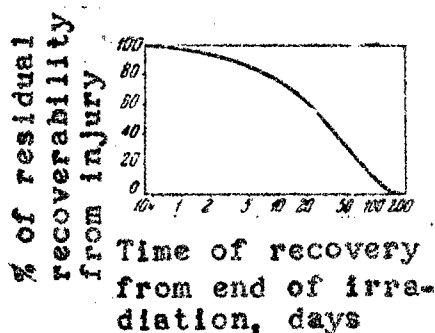

Fig. 3. Recovery After Irradiation.

Second case. Let us suppose that it will be necessary to stay in a contaminated area for two days for the purpose of carrying out emergency work. It has to be determined when this work can be begun.

Solution. The value of the X-factor remains the same, that is, 3.3. From a point on the abscissa axis which corresponds to 48 hours, let us erect a perpendicular to the point of intersection with the horizontal line drawn through a point on the ordinate axis corresponding to X = 3.3. Approximately at the site of intersection of these two perpendiculars we find a line which corresponds to t_1 = one day. Therefore, under these conditions (R_1 = 2000 r/hours; P = 4; D_e = 150 r) a 48-hour stay in the contaminated area will be possible one day after the blast.

Example 4. Let us assume that D_e = 50 r is taken as the effective dose for people living in an area of radioactive contamination. This dose produces changes in the blood but does not lead to the development of overt signs of radiation sickness. The average protection factor for people living a normal form of life is approximately equal to four. The intensity of radiation one hour after the blast was 800 r/hour. After how many days will it be possible to live in the contaminated area?

Solution. Let us find the value of the X-factor: X = R_1(P·D_e) = 800/(4X50) = 4. By means of a graph in Fig. 4 we find that a time equal to four days corresponds to the given value of the X-factor. With D_e = 100 r, this time will be equal to one day, and where D_e = 25 r, to 10 days. Therefore, living in this area of radioactive fallout

will be possible after one day, if D_e = 100 r; after four days, if D_e = 50 r; and after 10 days, if D_e = 25 r.
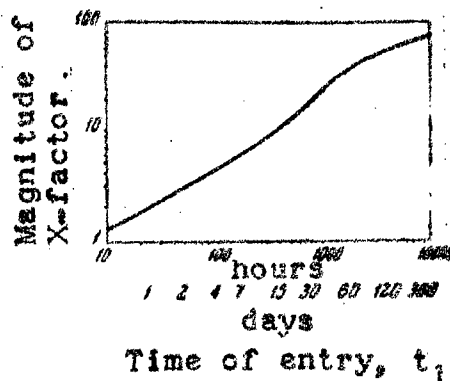

Fig. 4. Minimum Time After Which Living in a Contaminated Area Can Be Resumed Without Exceeding the Maximum Effective Dose. $X = R_1/P \cdot D_e$.

On the basis of the combination of data and the new methods worked out by the author, he drew a number of conclusions for planning radiological defense. The principal ones are the following:

1. For the purpose of evaluating the degree of radiation injury during the first four days after the blast the cumulative dose may be used. For later periods and for repeated brief irradiations the effective dose has to be used.

2. Many existing buildings (including the substructures of houses if the upper floors have not been essentially damaged) may provide a suitable degree of protection against radiation under conditions of adequate preliminary preparation for a prolonged stay there. Special shelters are potentially much better than houses or commercial buildings if they provide for a prolonged stay.

3. Brief irradiations with a relatively high radiation intensity are quite permissible during the performance of emergency tasks immediately after the attack.

4. In a severely contaminated area work may be resumed much earlier than has been calculated on the basis of radiation for "normal activity," keeping in mind the high protection factor of shelters and under the conditions that the personnel remaining continue to live in the shelters and that they travel in automobiles protected by sandbags.

N. Fedorov

Professor D. I. Zakutinskiy. Voprosy toksikologii radio-aktivnykh veshchestv. [Problems of the Toxicology of Radio-active Substances]. Moscow, 1959. 7.79 sheets.

The appearance of Professor D. I. Zakutinskiy's mono-graph devoted to general problems of the toxicology of radio-active isotopes is of indubitable interest.

In different portions of the monograph a general characterization is given of radioactive isotopes, the routes of entry and excretion are analyzed, the nature of distri-bution of them as well as the role of the physiological and physico-chemical factors in the distribution of the isotopes are examined; light is thrown on the characteristics of the effect of radioactive isotopes on the body, the clinical picture of the injuries, the principles of therapy of radi-ation injuries as well as the remote consequences and the effect of radioactive substances on the offspring. In addi-tion, information is presented concerning the maximum per-missible levels of concentration of certain radioactive sub-stances in the human body, water, air, methods of calculating the tissue doses created by β-emitting radioactive isotopes, brief data concerning the chemical, physical and toxicologi-cal properties of the radioactive isotopes which are most important in a practical sense (Ra^{226}, Rn^{222}, Po^{210}, Th^{224}, Ac^{231}, Pu^{239}, Sr^{89}, Sr^{90}, Cs^{137}, Y^{91}, Ce^{144}, Zr^{95}, Ru^{106}, Ru^{103}, H^3, Co^{60}, I^{131}, P^{32}, Na^{24}, Au^{198}). In the book there are indications of the need for using statistical methods of analysis of the results. Special attention is given to the method of Student, which has become very common and is frequently used in scientific investigations. Therefore, in his monograph the author analyzes a very extensive group of problems concerning the toxicology of radioactive isotopes.

Material concerning the remote consequences of injury by radioactive substances, their influence on the offspring and reactivity of the body are of considerable interest. In these sections the reader will find many interesting and important facts.

In the monograph there are a considerable number of disputable statements that are without adequate basis. Thus, for example, in analyzing the relationship of the biological effect of radioactive isotopes to the type of radiation the author writes: "If we take the toxicity of a γ-emitting sub-stance as unity, the β-emitters are 100 times more toxic, and the α-emitters are 100 times more toxic than the β-emitters or 10,000 times more toxic than the γ-emitters" (page 15). In this case we are dealing with the toxicity of radioactive substances which enter the body. As is well known, pure

γ-radiation is produced only by isomers of radioactive isotopes, with which, as far as we know, no toxicological experiments have been performed.

Unfortunately, the author does not present any specific experimental data on the basis of which he formulated this conclusion, which is of importance in principle. He does not even indicate how he derived his estimation of the relative effectiveness of the radioactive isotopes, whether by means of a comparison of their effectiveness when the data were expressed in microcurie units or on the basis of comparing the values of the tissue doses of radiation created by the radioactive isotope in the organs. It should be pointed out that numerous radiobiological investigations attest to approximately the same degree of biological effectiveness of X, γ-rays and β-rays.

On page 16 the author writes: "Investigations in the field of comparative toxicology of radioactive substances with different degrees of radiation energy show that the higher the energy of the particles the greater the toxicity possessed by the radioactive substance." In the author's opinion, what has been stated should be illustrated by Table 6. However, the material of this Table constitute an example of careless handling of factual material. In the Table under analysis the bulk of the material is presented inaccurately. First of all, the values of the lethal doses for H^3 are indicated incorrectly (0.4 μC/gram instead of 500-1000 μC/gram, as is correctly indicated on page 144). and for Ru^{106} (20 μC/gram instead of 8 μC/gram, as given in the data on page 144), of CS^{137} (8 μC/gram instead of 21 μC/gram). Secondly, effective quantitities of radioactive isotopes in a biological sense are not compared here. Thirdly, the radioactive isotopes are pointed out inaccurately and incorrectly distributed according to their β-particle energies. In the case of Sr^{90} and Ru^{106} the maximum β-particle energies (taking into consideration the β-radiation energy of their daughter radioactive isotopes Y^{90} and Rh^{106}, the effect of which certainly cannot be neglected in evaluating the effectiveness of the radioactive elements indicated) are not equal to 0.54 and 0.0392 MeV, but rather to 2.79 and 3.1 MeV respectively. It should also be noted that the maximum β-particle energy of Cs^{137} is not equal to 0.18 but rather to 0.52 MeV, as is correctly mentioned on page 141. Fourthly, the correct evaluation of the biological effectiveness of radioactive isotopes depending on physical (radiation energy; half-life period) and physiological (nature of distribution, rate of excretion) properties can be made only on the basis of a comparison of the values of tissue

85

doses of radiation created by β-emitters when they are introduced in equieffective quantities (in µC/gram). In consideration of the comments made above we are presenting data concerning the actual biological activity of the radioactive isotopes mentioned (according to the data of Yu. I. Moskalev, 1958).

Isotope	β-particle Energy, MeV	Values of the LD 50/30, µC/gram	"Mean Dose",[2] krep/gram
Ru^{106}	3.1^1	3.7	4.1
Sr^{90}	2.79^1	1.5	3.2
Cs^{137}	0.52	21.0	4.5
H^3	0.01795	1000	1.0

[1] Energy of mother and daughter isotopes.
[2] The "mean dose" is calculated by the assumption of a uniform distribution of the emitter with consideration of its excretion from the body.

The material presented constitute evidence to the effect that in reducing the β-radiation energy by approximately six times (Ru^{106} - Cs^{137}) the effectiveness of radioactive isotopes is unchanged. By comparison with a hard β-emitter (Ru^{106}) the soft β-emitter (H^3) was even more effective, because in the case of injury by equieffective quantities of these radioactive isotopes the "mean" tissue dose was four times less in the case of H^3. By comparing the effectiveness of radioactive isotopes in microcurie units Ru^{106} proved to 270 times more effective than H^3. However, such a comparison does not reflect the biophysical nature of the process. Gray (1947) emphasized the groundlessness and the error in comparisons made without consideration of physical rules and regulations underlying the biological effect of radiation.

In analyzing the degree of toxicity of radioactive substances depending on the route of their entry into the body D. I. Zakutinskiy indicates that their greatest toxicity is manifested when they enter the bloodstream directly; least, when they enter through the mouth (page 18). However, experimental material accumulated at the present time do not give us grounds for such a categorical assertion. This statement is justifiable only for radioactive isotopes which are poorly absorbed from the gastrointestinal

tract. The toxicity of radioactive isotopes which are completely absorbed from the intestine (for example, Cs^{137}, I^{131}) does not depend on the route of administration.

In the monograph crude experimental data are utilized which have not been properly treated. What has been stated applies primarily to certain results of research on the distribution of radioactive isotopes. At the present time, the results of experiments on the distribution of radioactive isotopes are customarily expressed in percentages of the quantity administered in the form of mathematical equations characterizing laws of accumulation and excretion of radioactive isotopes from the organs or in an extreme case in disintegrations per minute. The first two methods make it possible to evaluate quantitatively the results of experiments and compare with the observations of other authors. In the monograph many results of experiments on the distribution are presented in impulses per minute. As the result of different degrees of effectiveness of counter tubes, which change with time, different measurement geometries, etc., the results of the experiments expressed in impulses per minute are not comparable and may give only a qualitative characterization, and even that with great straining of the interpretation, of the behavior of a radioactive element in one organ or another. As an illustration let us present several examples. In Table 2 (page 11) material is given on the distribution of uranium fission products after inhalation. The results of the experiment are expressed in impulses. Here, even the time during which the given activity is shown has not been indicated. The reader has to guess whether the impulses are per minute or impulses per second. In addition, we do not find any indications of whether the table material characterizes the concentration of the isotope in the entire organ, one gram or one kilogram of the tissue either in the text or in the table; there are no data either concerning the species of animal on which the experiments have been performed. Finally, in the notes on Table 2 the radiochemical composition of the mixture of uranium fission products utilized has been indicated inaccurately (84.7 instead of 100 percent). In connection with the fact that it is mixture which is meant when the uranium fission products are mentioned later, this error with respect to the isotopic composition applies also to the other material on the toxicology of uranium fission products. The same comments may be made also with respect to Table 18 (page 89) and Table 22 (page 99).

In Table 8 (page 20-21) material is presented on the distribution of Co^{60} depending on the route of entry into

the body (administration through the mouth, subcutaneously, intravenously). In connection with the fact that the results are expressed in impulses per minute, it is hard to draw any conclusions, on the basis of the data presented, concerning the characteristics of distribution of this isotope depending on the route of administration, particularly since it is not known whether the same activity quantities were introduced into the animals. With this aim in view it would be better to take advantage of the results of research of other authors which makes it possible to draw quantitative conclusions. For this reason Figure 7 (page 34), which characterizes the dynamics of excretion of the uranium fission products, should not have been presented in the monograph.

In analyzing the problem of the characteristics of distribution of radioactive isotopes with their chronic entry into the body the author correctly points out that after a certain interval of time and because of the radioactive decomposition and excretion an equilibrium is established between the entry and concentration of the isotope in the body (page 22). The author's opinion that the time needed for establishing this equilibrium depends on the quantity of activity entering the body daily needs further experimental analysis, because the experimental data of other authors (E. B. Kurlyandskaya and coauthors, 1957) do not agree with this material. The author indicates that radioactive elements which are uniformly distributed (Cs^{137}, Na^{24}) are excreted in accordance with an exponential law. "The radioactivity of the urine and of the stool in this case characterizes the total radioactivity of the body" (page 36). The idea of the possibility of determining the total concentration of activity in the body according to the intensity of its excretion in the urine and stool should have been developed and made more specific, and the possible interrelationships between them should have been indicated.

The author's assertion that the kidneys are the main route of excretion of strontium after parenteral administration (page 141) is disputable. It is well known that after parenteral administration, as a rule, somewhat larger quantities of strontium and other alkaline earth elements are excreted through the gastrointestinal tract rather than through the kidneys. In analyzing the routes of excretion of radioactive substances the author directs attention to the fact that the majority of them is excreted chiefly through the gastrointestinal tract rather than through the kidneys, and he writes: "It is usually considered that this ratio is equal to 90:10." It probably should have been mentioned

that certain radioactive isotopes (Cs^{137}, Rb^{86}, K^{40}, I^{131}, Na^{24}) are excreted predominantly through the kidneys, regardless of the route of their entry, and the stool/urine ratio is equal to 1:9 rather than 9:1.

An abundance of interesting data has been presented in chapters characterizing the features of the biological effect of radioactive isotopes. However, even here we encountered disputable, inadequately reasoned-out postulates. A method of analyzing the biological effect of radioactive isotopes depending on the quantity of activity administered (page 39-40) suggested by the author may meet with objections. D. I. Zakutinskiy recommends distinguishing the following: 1) doses (better called activity quantities) producing an acute course of the process and death of the animal in two to three weeks; 2) doses producing a subacute course and death of the animal in one to four months; 3) doses causing death of the animal after 9-12 months; 4) doses causing the death of the animal at late periods, one to two years after the administration of the isotope and later; 5) doses causing remote consequences, including tumors; 6) maximum permissible doses. Only the doses of natural radioactive isotopes which are constantly maintained in the human body are absent from this list.

We consider it more accurate to distinguish acute, subacute and chronically effective quantities of radioactive isotopes. Considerable difficulties arise from attempts to use the division of doses proposed by the author with the aim of analyzing results of toxicological experiments on rats. The isotope in a quantity causing the death of the animal in 9 to 12 months and even after one or two years (third and fourth level doses) simultaneously possesses a tumor-producing effect and shortens life (fifth level of doses). These data indicate that the classification proposed is very artificial, and the third, fourth and fifth dose levels can be combined without any particular harm and can be regarded as chronically effective.

In analyzing the problem of the species sensitivity of animals to radioactive substances (Table 14, page 55), the author presents interesting data to the effect that after inhalation injury with radon mice, rats, and rabbits were more sensitive than dogs. This fact contradicts the data known in the literature to the effect that after the intravenous and intraperitoneal injection of radioactive isotopes (Ce^{144}, Cs^{137}, Y^{91}, $Sr^{89, 90}$) dogs are five to ten times more sensitive than rats (Yu. I. Moskalev, L. N. Budko, N. D. Borisova, V. N. Strel'tsova). Unfortunately, the author circumvents the problem of the causes of the

greater "radiosensitivity" of mice, rats, rabbits after inhalation of administration of radon by comparison with dogs and the lesser degree of sensitivity after the intravenous administration of the radioactive isotopes. By way of a working hypothesis the idea may be expressed that the more rapid extinction of rodents was not producted by the greater degree of radiosensitivity after the isotope was administered through the respiratory tract but rather is the result of a more active pulmonary ventilation, as a result of which, under otherwise equal conditions (same concentration of isotope and duration of exposure) considerably greater quantities of radon enter the bodies of small laboratory animals which produced the more rapid extinction of the rodents.

The author's ideas about the cumulative properties of radioactive isotopes and trace reactions have been developed with inadequate clarity. The important conclusion that under the conditions of repeated administration a marked increase is observed in the sensitivity of the body to radioactive isotopes (pages 40-41) should have been reasoned out with the use of specific data, particularly since experimental observations which exist do not give us the grounds for any such conclusion. Thus, for example, it is well known that under conditions of prolonged entry of radioactive isotopes into the body (Ru^{106}, Sr^{89}, Sr^{90}, Ce^{144}) the length of life may be shortened and the production of bone tumors and gastrointestinal tract tumors falls off sharply by comparison with a single administration of these quantities (L. A. Buldakov, Yu. I. Moskalev, V. N. Strel' tsova, Kuzma, Zander).

Referring to the research of S. P. Voskresenskiy and A. P. Novikova, the author points out that radioactive substances in every small doses have a harmful influence on the offspring in subsequent generations. Unfortunately, the magnitudes of the "very small quantities of radioactive isotopes" are not presented (page 41).

In the monograph considerable attention has been given to the effect of radioactive isotopes on the reactivity of the body to various drugs and preparations possessing a high degree of biological activity. In the radiobiological literature very few published investigations of this kind exist, particularly with respect to radioactive substances. Therefore, it can only be considered unfortunate that the author presents so few factual data and limits himself usually to references to the material of unpublished research. In individual cases, where factual data are presented, the material does not always make a convincing impression. Thus, for example, in Table 16 (page 84) data are

presented concerning the change in sensitivity of dogs to bromides and caffeine after the chronic effect of Sr^{90} (a year after stopping the administration. In connection with the fact that the isotope was administered for a long time (the duration of which, like the activity of the isotope, is not indicated either in the text or in the table), the sensitivity of the animals to these preparations might have changed in connection with the increasing age of the dogs, the effect of seasonal factors or the change in nutritional conditions. Therefore, for the purpose of evaluating the effect of Sr^{90} on the reactivity of animals to caffeine and bromides dynamic observations are needed not only on the experimental but also on control animals. Unfortunately, the results of experiments with control animals have not been presented either in the table or in the text.

In a chapter devoted to problems of toxicology of the individual elements (pages 136-144) there are discrepancies between the data in the literature and the data presented in the monograph. On page 141 the author writes that after the intravenous injection of strontium (it is not indicated whether Sr^{89} or Sr^{90}) in quantities of 0.3-0.5 mC/kg the animals (the species is not indicated: rats, dogs ?) die in 20-45 days. It should be noted that on page 16 (Table 6) different material has been presented attesting to the fact that after the injection of quantities of Sr^{90} two or three times as great (one μC/gram) the length of life of the animals was not 20-45 days but rather 129-144 days. According to numerous investigations, it may be asserted that the activity quantities of Sr^{90} presented on page 141 (the toxicity of Sr^{89} is less than that of Sr^{90}) cause the death of rats after the 200th day and of dogs during the first two weeks of the experiment. On pages 142 and 144 information is presented concerning the magnitudes of the acutely effective doses ($LD_{50/30}$) for yttrium and ruthenium, but it has not been indicated for which species of animals. As a result of considerable species differences in the radiosensitivity (for example dogs are five to ten times more sensitive than than rats), the latter is of more than a little importance. On page 138 it is not mentioned by what route of administration radiothorium (0.1-0.0001 mC/kg) causes the death of dogs at different intervals of time after the intoxication.

Among the minor defects we should like to present the following. In Table 10 (page 24) the time (in days) is not mentioned to which the picture of I^{131} distribution corresponds; radon is formed in the body as a result of the decomposition of radium, not of uranium (page 42); the term "tol-

erance concentration" is unfortunate (Table 13, page 53); on page 54 the unit of measurement (2 $\cdot 10^{-8}$?) has not been mentioned for the mixture of uranium fission products; in Table 17 (page 86) there are no data concerning the quantity of uranium fission products which were injected into rats; it is not clear how the author obtained breast milk from puppies on the first-seventh day after birth (!) (see Table 22, page 99). In the literature there is no information on how ethylenediaminetetraacetate accelerates Cs^{137} excretion from the body (page 115); it is not "hexamethylphosphate" but "hexametaphosphate" (page 115).

A number of minor slips attracts attention. Thus, for example, $LD_{50/15}$, $LD_{50/30}$ rather than LD_{50-15} and LD_{50-30} days should have been written (page 39, 137, 140, 142, 144, etc.); it should be "the physical basis of radiation" (page 149, fifthline from the top) rather than the "physiological basis". In Table 1 (page 5) the weight one μC of Sr^{89} as being $3.7 \cdot \cdot 10^{-13}$ grams is indicated erroneously (it should have been $3.7 \cdot 10^{-11}$ grams); the number of gram-atoms was given incorrectly for Bi^{210} ($4.1 \cdot 10^{-12}$ instead of $4.1 \cdot 10^{-14}$) and for Sr^{89} ($4.2 \cdot 10^{-15}$ instead of $4.2 \cdot 10^{-13}$); for Sr^{89} the number of atoms in one μC is given incorrectly ($2.6 \cdot 10^{9}$ instead of $2.6 \cdot 10^{11}$). In Table 20 (page 98) the duration of administration of the fission products and the magnitude of the total activity administered are not given for the chronic experiment; the term "average duration of the dose of isotope" (page 45) is unfortunate; in Tables 19-20 there are inadequate data concerning the survival rate and mortality rate of puppies born of control dogs, etc. It is difficult to analyze the material of Table 24 (page 107). There is no indication in it of the method of administration of the uranium fission products; it is not made clear what the carcass was, and no data are presented concerning the change in weight of the control rats in the remote periods. Evidently a loss of weight was also observed in the control rats shortly before death. If this is so the question arises: to what degree is this test specific for radiation injury?

A vexing error crept into the section of the monograph devoted to statistical methods of analysis of the results of toxicological experiments (pages 131-134). First of all, equation (4) for determining the parameter S (page 131) is written incorrectly. The third and fifth elements of this formula should have been presented in the form

$\dfrac{(\Sigma x)^2}{n_1}$ and $\dfrac{(\Sigma y)^2}{n_2}$ rather than $\dfrac{(\Sigma x)^2}{(n_1)}$ and $\dfrac{(\Sigma y)^2}{(n_2)}$, as was written in the text. As is well known, for the purpose of solving the problem of the reliability of differences between the experimental and control group (with the use of Student's method) special tables or graphs are used in which according to a given value of t and the number of observations n the value P is found which gives evidence of the reliability or lack of reliability of differences between the experimental and the control group. With this aim in view a table has been presented for determining the value P on pages 132-133 of the monograph. Unfortunately, this is an erroneously presented table which was used in another method (in the comparison of dispersions) of analysis of the results of experiments, which the author did not mention at all in the text.

A defect in the monograph is the absence of an author and subject index, which complicates the use of the monograph as a reference work on problems of toxicology of radioactive isotopes, particularly since the correlation between the content and the name of the chapters is incomplete. Thus, for example, in chapter 1, called "General Characteristics of Radioactive Substances," material is contained concerning the biological effect of them depending on their physical properties; in chapter 4, called "Excretion of Radioactive Substances from the Body," material is unexpectedly presented concerning the role of the direct and indirect influences in the development of intoxication after injury by radioactive substances (page 36); in chapter 5, called "Conditions Influencing the Nature of the Effect of Radioactive Substances," material is analyzed concerning the methods of calculating the tissue doses, on the maximum permissible levels of concentration of radioactive isotopes in the human body, etc.

In conclusion it should be noted that the work of Professor D. I. Zakutinskiy, which has been devoted to very important problems of modern science, is the first monograph on the toxicology of radioactive substances in the Soviet Union. This possibly also explains the defects noted in this criticism. It is to be hoped that they will be taken into consideration and corrected in the second edition. Despite the existing defects the book will be read with interest by everyone engaged in problems of toxicology of radioactive isotopes. The toxicologist will find extensive experimental material in this book which he can utilize in the performance of his research.

The book has been written in good literary language and can serve as a reference work on a number of problems of the toxicology of radioactive substances.

Yu. I. Moskalev

END

FOR REASONS OF SPEED AND ECONOMY

THIS REPORT HAS BEEN REPRODUCED

ELECTRONICALLY DIRECTLY FROM OUR

CONTRACTOR'S TYPESCRIPT